THE JOURNAL OF AGRICULTURAL SCIENCE

VOLUME IX 1918—19

Cambridge University Press
C. F. CLAY, Manager
LONDON: Fetter Lane, E.C. 4
also H. K. LEWIS & Co, Ltd, 136, Gower Street, London, W.C. 1
and WILLIAM WESLEY & SON, 28, Essex Street, London, W.C. 2
CHICAGO The University of Chicago Press
BOMBAY, CALCUTTA AND MADRAS. Macmillan and Co., Ltd.
TORONTO: J. M. Dent and Sons, Ltd.
TOKYO: The Maruzen-Kabushiki-Kaisha

CONTENTS

Part 1 (August, 1918)

Part 2 (April, 1919)

Part 3 (September, 1919)

Part 4 (October, 1919)

VOLUME IX AUGUST, 1918 PART I

BURIED WEED SEEDS.

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THE seeds of many species of plants have a habit of germinating in unexpected places under various conditions, and the question of the vitality of seeds is one that has frequently engaged the attention of scientists. It has often been claimed that seeds obtained from old pyramids and sepulchres have germinated when placed in favourable circumstances. but Becquerel[1] states that strict enquiry and experiment show that authentic seeds of such origin will *not* germinate, but that the seeds so obtained which do germinate have proved to be frauds inserted by the fellaheen for the sake of gain. Various writers (quoted by Becquerel) have claimed the power of germination for seeds buried for very long periods. Michelet claimed that seeds of *Galium anglicum*, buried 3000 years ago in the valley of Doubs, had retained their power of growth; von Heldreich, that *Glaucium Serpieri* from land covered by excavated scoriae 1500 years ago, was still viable; much doubt, however, has been thrown on the authenticity of such seeds.

Information of this kind is of comparatively little value as evidence, as there is no definite proof of the time at which the seed actually reached its position or of the way in which it was transported. More satisfactory evidence has been obtained by the attempted germination of seeds of known age by various observers. Becquerel quotes experiments in which 500 species of seeds were sown, some of which were nearly 200 years old. Only four families gave any germination, *Leguminosae*, *Nelumbiaceae*, *Malvaceae*, *Labiatae*.

Leguminosae. 18 species germinated out of 90 sown. Ages 28-87 years.
Nelumbiaceae. 3 species germinated. Ages 18-56 years.
Malvaceae. 1 species (*Lavatera pseudo-olbia*) germinated out of 15 sown. Age 64 years.
Labiatae. 1 species (*Stachys nepetaefolia*) germinated out of 14 sown. Age 77 years.

[1] Becquerel, P., "Recherches sur la vie latente des graines" *Ann. des Sci. Nat., Bot.* 1907, 5, pp. 193–311.

From 1840 to 1857 germination experiments were carried on by a committee appointed by the British Association, and the results were summarised in a series of reports. 220 species were very fully tested with the following results[1]:

Limit of years during which germinating capacity was retained	Number of species
43	1 (Leguminosae)
42	1 ,,
27	7 (5 Leguminosae, 1 Malvaceae, 1 Tiliaceae)
26	4
25	4
21	4
18	1
15	2
14	1
13	5
12	2
10	4
9	2
8	70
6	3
5	3
4	9
3	97

The table indicates that the majority of seeds retain their germinating capacity for comparatively few years, three years and eight years being the critical time for a large number of species. The seeds were kept under ordinary conditions of dry storage, and were representative of 67 natural orders. A few typical farm weeds were included:

	Years		Years
Hypericum hirsutum	3	Arctium Lappa	8
Plantago media	3	Daucus Carota	8
Conium maculatum	5	Nepeta Cataria	8
Fedia dentata	5	Rumex obtusifolius	8
Aethusa cynapium	8	Silene inflata	8
Anagallis arvensis	8	Vicia sativa	8

The length of time that seeds retain their germinating capacity has a very practical bearing on agriculture in connection with the vitality of the weed seeds that are buried in the soil and that are brought to the surface by cultivation. The conditions under which seeds exist when buried in the soil are utterly different from those of dry storage, and while some seeds are probably enabled to retain their germinating capacity for much longer periods when buried, others succumb at an early stage. Seeds in the soil are subject to fluctuating conditions of temperature, moisture,

[1] *British Association Reports*, 1850, pp. 160–168; 1857, pp. 43–56.

oxygen supply, etc. Becquerel[1] states that the resistance of seeds to low temperature depends solely on the quantity of water and gas in their tissues. If this quantity is sufficient the cold disorganises the protoplasm and nucleus, so killing the seeds, but if the protoplasm has attained by desiccation its maximum concentration and consequently its minimum of activity it completely escapes the action of low temperature and does not freeze, so that the seed retains its power of germination. If this be the case, immature seeds are likely to lose their power of germination quite soon after burial from the effects of cold, whereas mature seeds of the same species may be able to withstand the low temperature of winter for long periods of years. Vines[2] considered that long continued exposure to a not very low temperature proves fatal, and he stated that under ordinary circumstances starchy seeds retain their power of germination much longer than oily seeds. This latter statement is at variance with the commonly accepted idea, as it is usually considered that the oil in the seeds is of special assistance in the retention of vitality.

Under certain conditions the seeds of many species of plants are able to remain dormant in the soil for long periods, and to start into growth when they are brought to the surface by the processes of cultivation. The popular imagination often runs riot in this connection and stories are told of great crops of charlock, poppy and other weeds which appear when "old pasture" or "land which has never grown charlock before" is broken up. Careful enquiry usually shows that the "old pasture" was under tillage at no very distant date, or that the land that was supposed to be free from charlock has been ploughed rather more deeply than usual. Nevertheless, some evidence cannot be explained away thus, and much enquiry and experiment will be necessary before a full and satisfactory explanation is forthcoming. Similar reports come from other countries than England. Brulalette d'Abbeville[3] stated that alders appeared on some excavated soil although none had been known in the district for two centuries. According to Maquin-Tandon[3] soil that was thrown up in digging a canal at Toulouse was covered in two years with *Polypogon monspieliensis*, a plant which is lacking in Toulouse. Trochu[3] cites an instance in which sarrasin and millet came up when the soil was disturbed in an orchard which had been established for 10 or 12 years, the species having been originally sown as crops. In the same orchard, when trenches were dug to root prune the trees, heath and furze came up from

[1] *Loc. cit.*
[2] Vines, *Physiology of Plants.*
[3] Quoted by Becquerel, *loc. cit.*

seeds which must have been buried for 45 years, as at the time the field was cleared it was covered with these plants. When some new trees were planted 14 years later the same species reappeared, after burial for 59 years.

Peter[1] took samples of soil in forests of known anterior conditions at depths of 8, 16, and 25 centimetres. He found that the number of seeds which germinated diminished with the depth, and that at 25 centimetres the seeds were very scarce. Old forests only gave wood species, recent forests which had originally been prairie and field furnished some seeds characteristic of these situations.

The most abundant species were:

(1) *Juncus bufonius, J. conglomeratus, Sagina procumbens, Hypericum perforatum, Ranunculus repens, Plantago major, Gnaphalium uliginosum.*

(2) *Chenopodium polyspermum, Rubus Idaeus, Potentilla Tormentilla, Linaria Elatine, Centunculus minimus.*

Peter concluded that many of the seeds could retain their vitality for half a century.

Passerini[2] carried on germination experiments in pots with seeds of *Orobanche crenata* for 14 years, and found that the seeds lost practically all power of germination after lying in the soil for eight years.

Dorph-Petersen[3] carried out germination experiments with weed seeds in order to find out how long they retained their power of germination when buried in soil. *Thlaspi arvense, Sinapis arvensis, Geranium molle,* and *Malva vulgaris* often lay dormant for 6 12 years before germinating. It appeared that the rapidity of germination was affected by the length of time seeds were kept before planting, and also that ripe seeds had a higher germinating capacity and retained it longer than unripe seeds.

Pots containing 100 seeds of each of *Plantago lanceolata* and *Sinapis arvensis* were placed 12 ins. below the surface of ground in 1899, and each year after one pot was dug up and the seeds were allowed to germinate.

With *Plantago lanceolata* two-thirds of the seeds were dead by 1900 but 8 % still retained their germinating capacity after 10 years. In dry torage a similar lot of seeds kept their viability well for a few years, but all were dead in 10 years. With *Sinapis arvensis* the germinating

[1] Quoted by Becquerel, *loc. cit.*

[2] "Duration of vitality of seeds of *Orobanche crenata*," *Atti R. Accad. Econ. Agr. Georg. Firenze*, Series 7, 1910, 5, No. 1, pp. 1–7.

[3] *Jahresb. der Vereinigung für angewandte Botanik*, 1910. Summarised in *Journ. Board Agric.* 1911, pp. 599–600.

capacity was as high (87 %) after ten years as after one year, whereas in dry storage it was reduced to 82 % in one year and to 24 % in 10 years.

In these two cases it is evident that the conditions of burial are more conducive to the retention of germinating capacity than are those of dry storage. This provides a connecting link between the popular idea that charlock seeds can remain dormant in the soil for many years and the results obtained by the British Association Committee, in which species of Brassica kept in dry storage lost their power of germination in a very few years (*B. napus* eight years, *B. rapa* eight years, *B. oleracea* three years).

Dorph-Petersen also tested the effect of depth of burial by placing weed seeds 3 ins., 6 ins. and 12 ins. below the surface. The trials lasted for six years and showed that seeds placed at the greatest depths retained their germinating capacity best. The seeds of cultivated plants, especially grasses, died much more quickly in soil than did the related weed seeds.

Since August, 1915, experiments have been carried on at Rothamsted to test the power of germination of seeds buried in the soil under natural conditions at different depths, without any artificial placing or burial of seeds. To this end a number of samples were taken from different fields of known history by means of a sampling iron, 6 ins. by 6 ins. by 9 ins. This was driven into the ground, and the soil was carefully removed inch by inch, each inch being placed in a new paper bag and carefully labelled with the depth from which it was taken. The iron was driven far enough in to permit of sampling to a depth of 12 ins., and special precautions were taken that no crumbs of soil from the surrounding areas fell inside the sampling iron. The samples were then placed in clean sterilised pans or boxes in a greenhouse, kept watered, and left undisturbed for a time. The lower inches were chiefly heavy clay, which was broken up into small pieces by the fingers. After some months the sticky clay began to break down and disintegrate to some extent. Seeds soon began to germinate, and as soon as they were large enough to recognise they were noted and removed from the soil. Special care was taken that no plants were allowed to fruit and ripen seeds in the pans. Occasionally when the seedlings had been removed the soil was stirred and cut up with a knife. To avoid any danger of contaminating the samples, before the soil in any one box was interfered with all the surrounding boxes were covered with slates. As the boxes and pans were under cover in a greenhouse there was little danger of contamination by seeds carried on

the wind. As far as possible the ground in the immediate neighbourhood of the glasshouse was kept free from weeds and nothing was allowed to flower. After the experiment had been going on for some long time a fair number of *Senecio vulgaris* appeared in boxes in which they had not been evident in the early days of the test. Such seedlings were looked on with suspicion as being wind carried in all probability, and no account has been taken of them in the final results. After about 18 months a number of *Sonchus oleraceus* seedlings began to appear, and these also were considered as derived from wind carried seeds, as the species is so abundant in the immediate neighbourhood of the Laboratory. The conditions of the experiment, the types of the various seeds, and the distribution of the seedlings in the boxes render it highly improbable that any of the other seeds were of external origin, and it may be taken that the seedlings were derived from seed already in the soil when the samples were taken from the field.

Several samples were taken in each field selected for experiment and the fields were so chosen as to include land of as varied history as possible.

A. *Old Pasture* (never under arable as far as is known).
 (1) Harpenden Common. Sampled June 16th, 1916, 3 holes.
 (2) Park Grass. Sampled April 11th, 1916, 4 holes.

B. *Pasture, originally arable.*
 (1) Meadow at Laboratory house. Sampled April 28th, 1916, 4 holes.
 (2) Barn Field Grass. Sampled Oct. 19th, 1915, 4 holes.
 (3) Geescroft Field. Sampled about April 20th, 1916, 4 holes.
 (4) New Zealand Field (*a*). Sampled Aug. 24th, 1915, 4 holes.
 New Zealand Field (*b*). Sampled Sept. 24th, 1915, 4 holes.

C. *Arable Land.*
 (1) Long Hoos. Sampled April 25th, 1916, 2 holes.
 (2) Barn Field (8·0). Sampled April 6th, 1916, 2 holes.
 (3) Agdell (5). Sampled April 8th, 1916, 2 holes.

In the tables relating to each field the number of seeds which germinated at each inch are added together for all the holes sampled, except in the case of the totals, which are given for each individual hole for each species.

A. Old Pasture.

(1) *Harpenden Common.*

So far as is known this is genuine old pasture and has never been under tillage. The soil is of a fairly light nature and is deep, as the lowest inches

TABLE I. *Harpenden Common.* Holes 1, 2, 3.

		Depth in inches												Total for each hole	Grand Total
		1	2	3	4	5	6	7	8	9	10	11	12		
Arable weeds															
Atriplex or Chenopodium	Orache or Fat Hen	.	1	.	.	.	.	.	.	.	.	.	.	0 + 0 +1	1
Polygonum aviculare	Knot-grass	.	.		.	.	.	.	1	.	.	.	.	0 + 1 +0	1
	Total	.	1	.	.	.	.	.	1	.	.	.	.	0 + 1 +1	2
Grassland plants															
Achillea Millefolium	Yarrow	2	.	.	.	.	.	.	.	.	.	.	.	0 + 2 +0	2
Bellis perennis	Daisy	1	.	.		.	.	.	.	.	.	.	.	0 + 1 +0	1
Galium verum	Ladies' Bedstraw	4	.	.	.	.	.	.	.	.	.	.	.	0 + 0 +4	4
Luzula campestris	Field Woodrush	*	1	1	.	1	.	.	.	.	.	.	.	3* + * +*	3*
Vicia sp.	Vetch sp.	.	.	1	.	.	.	.	.	.	.	.	.	0 + 0 +1	1
	Total	7*	1	2	.	1	.	.	.	.	.	.	.	3* + 3* +5*	11*
Grasses															
Grasses, various		*	*	*	2	.	2	*	1	1	.	.	.	* + 6* – *	6*
Lolium perenne	Perennial Rye-grass	.	.	.	4	.	.	.	.	.	.	.	.	0 + 2 +2	4
Agrostis sp.	Bent-grass	.	2	3	1	.	.	4	.	.	.	.	.	5 + 2 +3	10
	Total	*	2*	3*	7	.	2	4*	1	1	.	.	.	5* + 10* + 5*	20*
Suspected intruders															
Senecio vulgaris	Groundsel	.	1	1	.	.	.	1	.	.	.	1	.	0 + 1 +3	4
Sonchus oleraceus	Common Sowthistle	1	.	.	.	.	.	.	.	.	.	.	.	0 + 0 +1	1
	Total	1	1	1	.	.	.	1	.	.	.	1	.	0 + 1 +4	5

* indicates the presence of an indefinite number of plants at some periods.

sampled consist of good soil, and not of clay. A large number of grass and Luzula (species, probably = *campestris*) seedlings appeared in the top inches of soil and grass seedlings were also abundant in the second and third inches, but below this depth very few seeds germinated, except that in one hole a considerable number of grasses appeared seven inches down about a year after the beginning of experiment. Isolated grass seedlings occurred down to the ninth inch, probably derived from seeds that had been washed through the crannies or carried down by worms. During the 14 months of the experiment only 10 other seedlings appeared, inclusive of all species, with the exception of a few suspected groundsel and sowthistle plants. Eight of these were typical grassland plants, but the *Atriplex* or *Chenopodium* in the second inch and the *Polygonum aviculare* in the eighth inch were probably derived from seeds carried by birds or stock, as sheep and cattle have free run of the common. It is rather surprising that so few species occurred other than grasses and woodrush, but this may be due to the fact that the grazing is so close that only a very small proportion of plants have any chance to ripen and shed their seeds. The grasses that appeared were varied, but the greater number consisted of species of Agrostis, especially below the top inch of soil. Clovers were conspicuous by their absence, although plenty of leguminous plants occur on the Common. This is again attributed to the failure of the plants to form seed except in isolated cases.

(2) *Park Grass.*

There is no evidence available of this land being ploughed, and it is quite certain that it has been under grass for at least 300 years. The soil is fairly heavy and carries a good natural herbage, which has been allowed to develop undisturbed by stock for 40 years or more. Hay is cut every year, but as many of the plants have the opportunity of ripening their seeds one would naturally expect a much greater variety of species than was obtained from the close cropped Common soil. The difference proved to be most striking, for, apart from grasses, leguminous plants and possible intruders, 353 seedlings appeared between April, 1916, and August, 1917, compared with less than 20 from the Common soil. Practically all these were typical grassland plants[1], and the greater number occurred in the top six inches of soil. The maximum yield of seedlings was obtained from the second inch, and the number steadily

[1] *Centaurea nigra, Cerastium vulgatum, Ranunculus* sp. and *Stellaria media* have been separated out in the other tables as arable or grassland plants, but here they are so obviously present in their capacity of grassland plants that no division has been made in Table II.

TABLE II. *Park Grass.* Holes 1, 2, 3, 4.

		Depth in inches 1	2	3	4	5	6	7	8	9	10	11	12	Total for each hole	Grand Total
Arable weeds															
Brassica alba	Charlock				1									1 + 0 + 0 + 0	1
Atriplex patula	Orache				1			1						0 + 0 + 0 + 2	2
Atriplex or Chenopodium	Orache or Fat Hen						1							0 + 1 + 0 + 0	1
	Total				2		1	1						1 + 1 + 0 + 2	4
Grassland plants															
Achillea Millefolium	Yarrow	1												0 + 0 + 1 + 0	1
Ajuga reptans	Bugle	5	56	40	17	12	4	3	5	1	5			8 + 15 + 103 + 22	148
Carex sp.	Sedge								1					0 + 0 + 0 + 1	1
Centaurea nigra	Hardhead	7	20		1	4								0 + 30 + 1 + 1	32
Cerastium vulgatum	Mouse-ear Chickweed	2	3	1	4	2	2			1	1			2 + 0 + 2 + 12	16
Conopodium denudatum	Pignut	1	16	5	3									2 + 5 + 15 + 3	25
Luzula campestris	Field Woodrush		4	10	3	6	2				1			0 + 19 + 6 + 1	26
Potentilla fragariastrum	Barren Strawberry		3		1									0 + 0 + 0 + 4	4
Prunella vulgaris	Selfheal	3			1									0 + 0 + 3 + 1	4
Ranunculus acris	Tall Buttercup						1				1			0 + 1 + 1 + 0	2
" bulbosus	Bulbous Buttercup					1		1						0 + 0 + 0 + 2	2
Rumex acetosa	Sorrel	4	6	1	2									8 + 1 + 3 + 1	13
Stellaria graminea	Heath Stitchwort	1		5	1									0 + 1 + 1 + 5	7
" media	Chickweed			1										0 + 1 + 0 + 0	1
Veronica Chamœdrys	Germander Speedwell	6	25	15	11	8	3	1	1					0 + 16 + 23 + 31	70
" sp.	Speedwell sp.									1				0 + 0 + 0 + 1	1
Lotus corniculatus	Birdsfoot Trefoil		1	2	2			1						0 + 6 + 0 + 0	6
Trifolium pratense	Red Clover				1	1								0 + 1 + 1 + 0	2
" repens	White Clover	1	1			2	1			1	1		1	5 + 2 + 1 + 0	8
" (cut-leaved)	Clover sp.		1					1			1			1 + 2 + 0 + 0	3
	Total	31	136	80	47	36	13	7	7	4	10		1	26 + 100 + 161 + 85	372
														Less Leguminosae	353
Grasses															
Agrostis alba	Bent-grass							1						0 + 0 + 0 + 1	1
Anthoxanthum odoratum	Sweet Vernal-grass	2	2	1	1								1	2 + 3 + 2 + 0	7
Holcus lanatus	Yorkshire Fog		3	8		1								0 + 10 + 2 + 0	12
Lolium perenne	Perennial Rye-grass	1	1											1 + 1 + 0 + 0	2
Poa pratensis	Smooth-stalked Meadow-grass	1												1 + 0 + 0 + 0	1
Grasses, various		3*	*	1*	*		1	1						1* + 1* + 4 + 0	6*
	Total	7*	6*	10*	1*	1	1	2						5* + 15* + 8 + 1	29*
Suspected Intruders															
Senecio vulgaris	Groundsel	1	1			2		1			1			1 + 2 + 1 + 2	6
Sonchus oleraceus	Common Sowthistle	1			1					1				2 + 1 + 0 + 0	3
	Total	2	1		1	2		1		1	1			3 + 3 + 1 + 2	9

declined to the sixth inch, below which depth very few seeds germinated, although individuals appeared right down to the stiff clay of the twelfth inch. Four arable seedlings put in an appearance, but it is possible that they had been carried by birds or had been brought in on the feet of men or horses and had then worked their way down into the soil. The distances from the sampled area to the nearest arable field is considerable, and neither charlock, fat hen nor orache have seeds that are at all well adapted for wind dispersal. Fat hen and charlock seeds are heavy and could not travel on the wind, and the orache remains enclosed in its envelope, while the weight of the seed is such as to render it improbable that the wind plays any effective part in dispersing this weed, as the envelope is not efficiently winged. The large number of *Ajuga* seedlings obtained from one hole six inches square is remarkable—103 plants of one species from such a small area being the second greatest number obtained during the investigation. It is obvious that the sample must have been taken from an area on which the plant is thoroughly well established as the seeds were found right down to the tenth inch. The same remark applies to *Veronica Chamaedrys*, though in this case the seeds were more evenly distributed over three holes, while the fourth hole was utterly barren of speedwell. *Conopodium* did not appear at all until the experiment had been running for nearly a year, and it was very difficult to be certain whether many of the tiny plants were genuine seedlings or whether they had arisen from very small tubers which were already in the soil. Even the smallest plants with obvious cotyledons developed their tubers at an early stage, but probably the 25 plants observed included both true seedlings and small older plants. The late appearance was probably due to the fact that *Conopodium* is naturally a very early plant and as it was later than the natural time of germination and growth when the experiment was set up the seeds lay dormant for a year till the usual growing period came round again. *Centaurea nigra*, again, gave rise to a certain amount of doubt at first, but it is probable that nearly, if not quite, all the 32 plants were true seedlings. In this case again nearly all the seedlings arose from one hole, indicating a local accumulation of seeds. The leguminous plants were not very well represented, as only 19 individuals appeared. This may be because the hay is usually cut before many of the clover seeds are ripe, so that the ultimate effect of haying on these species approximates somewhat to that of grazing, as seen on the Common.

B. PASTURE, ORIGINALLY ARABLE.

(1) *Laboratory House Meadow*[1].

The field was originally under arable cultivation, but in 1856 was fenced in and was sown with barley and grass. The grass seeds failed at first, and it was not until 1859 that a successful stand was obtained. Since that date the field has been kept as a meadow and mown for hay, no stock being run on it.

This field has been down to grass longer than any other area considered in this experiment, with the exception of the permanent pasture of the Park and Common. Although 58 years have elapsed since the land was under the plough a certain number of arable seedlings appeared from the soil samples, and the number is large enough to lead one to assume that a considerable proportion must have been buried in the soil from the time of grassing down. *Atriplex patula, Polygonum aviculare,* and *Veronica Tournefortii* occurred in two or three of the holes to a depth of several inches, and it is most improbable that so many well distributed seeds should have been introduced by external agency and have worked their way through the grass carpet down through the soil. It is more difficult to draw any conclusion with regard to those species which appeared in one single hole or as isolated individuals. *Arenaria serpyllifolia, Polygonum Convolvulus, Brassica* sp. and *Matricaria inodora* were represented by single plants, but as the seeds all occurred at a considerable depth, and as none of them are at all well adapted for dispersal over any considerable area, it is quite possible that they represent survivors from long buried seeds. On the other hand *Papaver* sp. and *Sonchus asper* appeared only in the first and second inches, and as both these species are easily carried by the wind, the seeds were almost certainly introduced in these cases. *Anagallis arvensis* and *Alchemilla arvensis* are doubtful species. *Anagallis* appeared from three holes from three to seven inches below the surface, which is an argument for the seeds being survivors of widely distributed long buried seeds, but as the species is most abundant in the allotments close by an element of doubt must be admitted. Four *Alchemilla arvensis* seedlings appeared from one hole, three of them being in the second inch, which suggests that by some accident a fruiting plant or a number of seeds were introduced at the spot, so that the seeds might be of more recent date.

[1] J. B. Lawes, "The history of a field newly laid down to permanent grass," *Journ. Roy. Agric. Soc. Eng.* 1889, **25**, pp. 1–24.

TABLE III. *Laboratory House Meadow.* Holes 1, 2, 3, 4.

		Depth in inches: 1	2	3	4	5	6	7	8	9	10	11	12	Total for each hole	Grand Total
Arable Weeds															
Alchemilla arvensis	Lady's Mantle		3			1								0 + 0 + 4 + 0	4
Anagallis arvensis	Poor Man's Weather-glass			2	1			1						2 + 0 + 1 + 1	4
Arenaria serpyllifolia	Thyme-leaved Sandwort					1								1 + 0 + 0 + 0	1
Atriplex patula	Orache			2	3	5								6 + 2 + 0 + 2	10
Brassica sp	Charlock								1					0 + 0 + 0 + 1	1
Matricaria inodora	Scentless Mayweed					1								0 + 0 + 1 + 0	1
Papaver sp.	Poppy	1												1 + 0 + 0 + 0	1
Polygonum aviculare	Irongrass, Knotweed, Knot-grass		1	1	1									0 + 1 + 2 + 0	3
,, Convolvulus	Black Bindweed			1										0 + 0 + 1 + 0	1
Sonchus asper	Corn Sowthistle	1	1											0 + 2 + 0 + 0	2
Veronica Tournefortii	Large Field Speedwell		1			1								0 + 0 + 1 + 1	2
	Total	2	6	6	5	9		1	1					10 + 5 + 10 + 5	30
Arable or Grassland plants															
Capsella Bursa-pastoris	Shepherd's Purse	1												1 + 0 + 0 + 0	1
Centaurea nigra	Hardhead	2	1	1	1									2 + 0 + 3 + 0	5
Cerastium vulgatum	Mouse-ear Chickweed	3	11	11	6	1	1							8 + 17 + 6 + 2	33
Geranium sp.	Geranium, Cranesbill		1											1 + 0 + 0 + 0	1
Plantago lanceolata	Ribwort Plantain	1												0 + 0 + 1 + 0	1
,, major	Greater Plantain		1	1	1									2 + 0 + 0 + 1	3
Ranunculus bulbosus	Bulbous Buttercup	1					1							0 + 0 + 0 + 2	2
,, sp.	Buttercup sp.				1									0 + 1 + 0 + 0	1
Stellaria media	Chickweed			2										0 + 2 + 0 + 0	2
	Total	8	14	15	9	1	2							14 + 20 + 10 + 5	49
Grassland plants															
Bellis perennis	Daisy	2	3											5 + 0 + 0 + 0	5
Chrysanthemum leucanthemum	Ox-eye Daisy			2										2 + 0 + 0 + 0	2
Galium verum (not seedlings)	Ladies' Bedstraw	3*	9											12* + 0 + 0 + 0	12*
Prunella vulgaris	Selfheal			1										1 + 0 + 0 + 0	1
Rumex Acetosa	Sorrel	1												1 + 0 + 0 + 0	1
Trifolium repens	White Clover	6	6	8	6									12 + 7 + 5 + 2	26
,, (cut-leaved)	Clover sp.	2		4										4 + 2 + 0 + 0	6
Veronica serpyllifolia	Thyme-leaved Speedwell	13	9	5	6	2								26 + 1 + 0 + 8	35
,, sp.	Speedwell sp.	8	4											12 + 0 + 0 + 0	12
	Total	35*	31	20	12	2								75* + 10 + 5 + 10	100*
Grasses															
Agrostis sp.	Bent-grass				1								1	1 + 1 + 0 + 0	2
Lolium perenne	Perennial Rye-grass	1	1	4	6	1		5			1			1 + 3 + 10 + 5	19
Poa pratensis	Smooth-stalked Meadow-grass	7	12	32	17		2		1		2		1	27 + 22 + 23 + 2	74
Poa sp.	Meadow-grass sp.		17	6	1		4	1						0 + 0 + 14 + 15	29
Grasses, various		*	2*	1*	2	3		1	3		1		1	2* + 4* + 5* + 3*	14*
	Total	8*	32*	43*	27	4	6	7	4		4		3	31* + 30* + 52* + 25*	138*
Suspected intruders															
Lactuca sp.	Lettuce (garden)								1					0 + 0 + 0 + 1	1
Senecio vulgaris	Groundsel	3	4	2	1	2		3	1					3 + 4 + 2 + 7	16
Sonchus oleraceus	Common Sowthistle		1		1	1								0 + 0 + 2 + 1	3
	Total	3	5	2	2	3		3	2					3 + 4 + 4 + 9	20

The typical grassland plants and those common to arable or grassland occurred in some number, *Cerastium vulgatum*, *Trifolium repens* and *Veronica serpyllifolia* being the most abundant. All these seeds were segregated towards the surface, most of them being in the first three inches and none at all appearing below the sixth inch. Nearly all the true arable species were in the third, fourth and fifth inches with representatives in the seventh and eighth inches, which furnishes an additional proof that they are genuine buried seeds, as if aliens were introduced to any extent one would expect to find them in the upper inches as well in process of making their way down, as happened with *Papaver*, *Sonchus* and the doubtful *Alchemilla*. Grass seeds were abundant, chiefly in the top three inches, though viable seeds were found right down to and including the twelfth inch.

(2) *Barn Field Grass.*

This field was under ordinary farm cultivation till 1874, after which it was laid down to grass. As it was not an experimental field the earlier history is not very clear, but probably barley was the last crop carried prior to grassing down. Very few true arable weeds appeared in the pans, 12 seedlings of four species making up the entire crop. Clover and miscellaneous species characteristic of grassland were fairly abundant, but again very few species were represented, only 10 varieties appearing, of which three were clovers. Field observations showed that the herbage of the field now consists of grasses and clover with very few other species, so that it follows naturally that the species of buried weed seeds should be equally few in number. The fact that most of the arable seeds occur below the fifth inch of soil affords another proof that they were originally present and that they have not been gradually introduced by some external agency such as wind and animals. A few seeds may be introduced occasionally as the field is stocked with cattle, but the probability is that most of these die off before they have any chance of being washed down through the dense grass covering of the soil. If any contamination from outside sources were going on, one would expect a larger number of arable seeds to occur near the surface, as they would take some considerable time to be carried down five inches, but as a matter of fact only 1 such seedling appeared in the upper five inches. *Trifolium repens*, *Cerastium vulgatum* and *Ranunculus bulbosus* were present in large numbers and accounted for 91 % of the grassland plants. Nearly all these seeds occurred in the top six inches of soil, as was also seen in Geescroft, whereas all but one of the arable weeds appeared from six to eleven inches

TABLE IV. *Barn Field Grass.* Holes 1 E, 2 E, 1 W, 2 W.

		Depth in inches												Total for each hole	Grand Total
		1	2	3	4	5	6	7	8	9	10	11	12		
Arable weeds															
Atriplex patula	Orache							3		4				0+ 0+ 5+ 2	7
Brassica alba	Charlock						2					1		0+ 0+ 3+ 0	3
Linaria minor	Small Toadflax						1							1+ 0+ 0+ 0	1
Papaver sp.	Poppy sp.		1											0+ 1+ 0+ 0	1
	Total		1				3	3		4		1		1+ 1+ 8+ 2	12
Arable or Grassland plants															
Cerastium vulgatum	Mouse-ear Chickweed	9	10	4	2	3	6		1	2		1	7	3+ 3+24+15	45
Myosotis arvensis	Field Forget-me-not				1									0+ 1+ 0+ 0	1
Ranunculus bulbosus	Bulbous Buttercup	1	5	19	7	4	2	4			3		1	8+24+ 3+11	46
Stellaria media	Chickweed			1							1			0+ 1+ 0+ 1	2
	Total	10	15	24	10	7	8	4	1	2	4	1	8	11+29+27+27	94
Grassland plants															
Bellis perennis	Daisy	3		3	1			2						6+ 3+ 0+ 0	9
Rubus sp.		1		1										1+ 1+ 0+ 0	2
Rumex sp.	Dock sp.			1	2	2								0+ 1+ 4+ 0	5
Trifolium pratense	Red Clover				1									0+ 1+ 0+ 0	1
,, repens	White Clover	47	29	27	12	3	5	4	2	1		1		43+14+31+43	131
,, (cut-leaved)	Clover sp.	1												0+ 0+ 1+ 0	1
	Total	52	29	32	16	5	5	6	2	1		1		50+20+36+43	149
														Less Leguminosae	16
Grasses															
Agrostis alba	Bent-grass	22	11	16	3	1	1	2	1		1			0+ 5+14+39	58
Alopecurus pratensis	Foxtail	4	1											0+ 0+ 0+ 5	5
Avena pubescens	Downy Oat				1									0+ 0+ 1+ 0	1
Festuca ovina	Sheep's Fescue	1		1	1									1+ 1+ 0+ 1	3
Holcus lanatus	Yorkshire Fog	5	10	6	1		1							0+ 1+ 9+13	23
Lolium perenne	Perennial Rye-grass	31	26	22	19	7	7	1	2				1	22+23+41+30	116
,, ,, *or* Poa annua	,, ,, *or* Annual Meadow-grass	13	4	6	2									0+25+ 0+ 0	25
Poa annua	Annual Meadow-grass		3											0+ 0+ 3+ 0	3
Triticum repens	Couch-grass			1				1						0+ 1+ 0+ 1	2
Grasses, various		4	3	2	1			2	1				1	6+ 3+ 3+ 2	14
	Total	80	58	54	28	8	9	6	4		1		2	29+59+71+91	250
Suspected intruders															
Senecio vulgaris	Groundsel	1	1			1		1	1	1	1	2	1	5+ 4+ 1+ 0	10
Sonchus oleraceus	Common Sowthistle		3									1		2+ 0+ 1+ 1	4
	Total	1	4			1		1	1	1	1	3	1	7+ 4+ 2+ 1	14

below the surface. The field is sometimes stocked with cattle and sometimes cut for hay, and the plants have opportunities of ripening their seeds, as cattle do not eat the herbage down so seriously as sheep. This helps to account for the comparatively large number of grasses and other grassland plants, the grasses being most abundant in the top four inches, the abundance being in sharp contrast to the paucity of arable weed seeds. The scarcity of arable plants may either be due to the length of time the seeds have been buried or to the fact that comparatively few seeds were left in the soil at the time of grassing down. This last suggestion is made because (as will be seen later) the adjoining arable field also yields remarkably few seeds, and the two fields were originally part of the same area. Certainly the 12 arable seedlings from this field compare very badly with the 75 plants obtained from a similar area in Geescroft. On the other hand Barn Field has been definitely under grass since 1874, while Geescroft was not finally thrown into the Park till 1885, and the extra eleven years would possibly account for the destruction of a large number of arable seeds that may have been originally present in the soil.

(3) *Geescroft Field.*

This area was originally under arable cultivation, and was used for various manurial experiments until 1878, but the land was very damp and difficult to work and frequently became waterlogged. It was left fallow for three years and in 1882 an unsuccessful attempt was made to seed it down to grass. Barley and clover were then cropped but in 1885 most of the field was thrown into the Park, so that the area has been under grass since that date. Geescroft is a very long way from any ploughed land and it is also protected to some extent by belts of trees, so that it is most improbable that it has been infected with arable seeds by means of wind carriage. It may safely be assumed that practically all the seedlings of arable weeds that came up in this experiment were derived from seeds that were lying dormant in 1885 or that were produced from plants that may have managed to survive for a year or two when the land was put down to grass. Arable weed plants very soon succumb to the conditions obtaining when land is under grass, and it is therefore unlikely that many of the seeds are of later date than 1885.

74 seedlings of typical arable weeds appeared in the soil of samples aggregating one foot in area, the number of each species varying from 1—6, except in the case of *Polygonum aviculare*, of which 52 plants appeared. These *Polygonum* seedlings occurred in every inch except the first, the largest numbers appearing from six to nine inches down. The

TABLE V. *Geescroft Grass.* Holes 1, 2, 3, 4.

		Depth in inches													
		1	2	3	4	5	6	7	8	9	10	11	12	Total for each hole	Grand Total
Arable weeds															
Anagallis arvensis	Poor Man's Weather-glass	.	.	.	.	.	.	1	.	1	.	1	.	1 + 0 + 2 + 0	3
Arenaria serpyllifolia	Thyme-leaved Sandwort	.	.	.	.	1	.	.	.	.	.	.	1	0 + 0 + 0 + 2	2
Atriplex patula	Orache	.	.	.	.	3	.	2	.	.	.	.	.	0 - 1 + 1 + 3	3
Caucalis?		.	.	.	.	1	1	.	2	2	.	.	.	0 + 2 + 0 + 4	6
Papaver sp.	Poppy sp.	.	.	.	.	1	.	.	.	.	.	1	1	0 + 0 + 0 + 3	3
Polygonum aviculare	Knot-grass	.	2	3	4	3	13	9	6	6	2	3	1	9 + 18 + 6 + 19	52
,, Convolvulus	Black Bindweed	.	.	.	1	.	.	.	.	.	.	.	.	0 + 1 + 0 + 0	1
Veronica (agrestis?)	Field Speedwell	1	.	.	.	.	.	.	.	.	.	.	.	0 + 1 + 0 + 0	1
Viola tricolor	Wild Pansy	.	.	.	.	.	.	.	.	.	.	1	.	0 + 0 + 0 + 1	1
	Total	1	2	3	5	9	14	12	8	9	2	6	3	10 + 23 + 9 + 32	74
Grassland or arable plants															
Centaurea nigra	Hardhead	.	.	.	.	.	1	.	1	.	.	.	.	2 + 0 + 0 + 0	2
Cerastium vulgatum	Mouse-ear Chickweed	2	8	.	3	.	.	.	.	.	.	.	.	0 + 0 + 2 + 11	13
Myosotis arvensis	Field Forget-me-not	.	1	.	.	.	.	.	.	.	.	.	.	1 + 0 + 0 + 0	1
Plantago major	Greater Plantain	7	7	5	2	2	2	1	2	.	.	.	.	2 + 4 + 9 + 13	28
Stellaria media	Chickweed	.	1	1	.	.	.	.	1	.	.	1	.	0 + 2 + 0 + 2	4
Taraxacum vulgare	Dandelion	.	1	.	.	.	.	.	.	.	.	.	.	0 + 0 + 1 + 0	1
Viola sp.	Violet	.	.	1	.	.	.	.	.	.	.	.	.	1 + 0 + 0 + 0	1
	Total	9	18	7	5	2	3	1	4	.	.	1	.	6 + 6 + 12 + 26	50
Grassland plants															
Bellis perennis	Daisy	5	9	1	.	1	.	.	.	.	1	.	.	10 + 2 + 0 + 5	17
Conopodium?	Pignut?	.	.	.	.	1	.	.	.	.	.	.	.	0 + 0 + 0 + 1	1
Luzula campestris	Field Woodrush	2	1	2	.	.	.	.	.	.	.	.	.	1 + 4 + 0 + 0	5
Ranunculus acris	Tall Buttercup	3	.	.	.	.	.	1	.	.	.	.	.	0 + 0 + 2 + 2	4
,, ,, *or* R. repens	Tall *or* Creeping Buttercup	2	1	.	.	1	.	.	.	.	.	.	.	0 + 0 + 4 + 0	4
,, bulbosus	Bulbous Buttercup	.	1	1	.	1	.	.	1	.	.	.	.	1 + 1 + 1 + 1	4

Rubus sp.		1	.	1	.	.	.	.	.	.	.	.	.	0 + 0 + 1 + 1	2
Rumex Acetosa	Sorrel	.	1	1	.	.	.	.	.	.	.	.	.	2 + 0 + 0 + 0	2
Urtica dioica	Stinging-Nettle	.	.	1	.	.	.	.	.	.	.	.	.	0 + 0 + 1 + 0	1
Veronica serpyllifolia	Thyme-leaved Speedwell	.	4	2	1	.	.	.	.	.	.	.	.	3 + 0 + 0 + 4	7
Trifolium pratense	Red Clover	1	.	.	.	.	1	.	.	.	1	.	.	2 + 1 + 0 + 0	3
„ repens	White Clover	8	26	37	15	1	4	6	8	2	3	2	2	25 + 39 + 27 + 23	114
„ (cut-leaved)	Clover sp.	2	9	12	.	3	1	1	2	2	1	1	.	12 + 11 + 2 + 9	34
	Total	24	52	58	16	8	6	8	11	4	6	3	2	56 + 58 + 38 + 46	198
														Less Leguminosae	47
Grasses															
Agrostis sp.	Bent-grass	21*	14	7	7	.	1	1	.	.	.	.	1	16 + 18 + 5 + 13*	52*
Arrhenatherum avenaceum?	False Oat?	.	1	.	.	.	.	.	.	.	.	.	.	0 + 0 + 0 + 1	1
Festuca ovina	Sheep's Fescue	.	.	1	.	.	.	.	.	.	.	.	.	0 + 1 + 0 + 0	1
Holcus lanatus	Yorkshire Fog	2	2	6	.	.	2	1	.	.	1	.	.	3 + 2 + 4 + 5	14
Lolium perenne	Perennial Rye-grass	1	2	4	1	.	1	2	.	1	.	1	.	1 + 2 + 7 + 3	13
Poa pratensis	Smooth-stalked Meadow-grass	2	4	2	.	.	.	.	.	.	.	.	.	1 + 0 + 7 + 0	8
Poa sp.	Meadow-grass sp.	.	.	3	1	1	1	.	.	.	.	.	.	0 + 4 + 2 + 0	6
Grasses various		8*	*	4	4	3	3	2	5	.	3	2	1	5* + 3 + 11* + 16	35*
	Total	34*	23*	27	13	4	8	6	5	1	4	3	2	26* + 30 + 36* + 38*	130*
Suspected intruders															
Senecio vulgaris	Groundsel	.	4	.	3	2	.	1	.	.	3	3	.	6 + 3 + 3 + 4	16
Sonchus oleraceus	Common Sowthistle	2	.	.	1	.	.	1	1	1	.	.	.	2 + 2 + 0 + 2	6
	Total	2	4	.	4	2	.	2	1	1	3	3	.	8 + 5 + 3 + 6	22

seedlings were well distributed in the soil from each of the four sampled holes, indicating that the mother plants were probably common over the field and were not localised in a few places. It is surprising that such a large number of seeds have retained their germinating capacity for over 30 years and the survival of so many suggests that *P. aviculare* was exceedingly plentiful when the area was cultivated. The old Rothamsted records show that this was actually the case and that knotgrass was one of the worst weeds occurring among the crops. *Plantago major* was also very characteristic at that time and a number of seedlings appeared during the present experiment, but as this species is common to both arable and grass land and still occurs in the field, it is not possible to ascertain the length of time the seeds had been buried in the soil. Those occurring in the upper layers of soil are nearly certain to be of comparatively recent date. None of the typical arable weeds that appeared in the soil samples are to be found in the surface vegetation of the field nowadays, whereas they were all recorded as being present among the crops in 1867, a fact which renders it still more probable that the seedlings obtained in the experiment grew from seeds which had been buried in the soil at least since the land was thrown into the Park in 1885.

A large number of typical grassland seedlings appeared and also a certain number of species that are common to both arable and grassland. The greater number (75 %) of the grassland seeds were present in the top four inches of soil, whereas most of the arable seeds (68 %) were found from five inches to nine inches below the surface. The stock of grassland seeds is replenished year by year as the plants on the surface ripen their fruits, so that it naturally happens that the bulk of the youngest and most viable seeds occur near the surface. As the seeds get carried down into lower depths in the course of time many of them lose their power of germination with increasing age, and the lowest inches contain very few seeds that are capable of growth when the soil is disturbed. On the other hand the long-buried arable seeds have apparently found some conditions of equilibrium in the middle depths which have enabled them to remain unharmed all these years. When the land first passed out of cultivation the greater number of arable weed seeds must have been in the top few inches. The conditions of aeration, temperature and moisture down to a depth of three or four inches at least, are such as to induce germination, which is effective or incipient according to the variety of seed concerned and the depth at which it is buried. Consequently in the course of the first few years most of the seeds towards the surface died off, either by germination or by rotting. The more deeply buried seeds,

however, did not find the conditions suitable for germination, and therefore lay dormant. Many of them must have died off, but some seeds were apparently at such a nice condition of maturity that they were able to establish equilibrium with their surroundings and so have survived in a condition of latent life or dormancy until the present time, 30 years after they were shed.

(4) *New Zealand Field.*

This field was under grass from 1906 till 1915. Prior to 1906 it was under arable cultivation and in the autumn of 1915 it was again ploughed up and is now cropped in the ordinary course of farm management. The soil samples were taken before the grass was disturbed and two separate sets of samples from four holes each were taken in August and September 1915. As the field is now cropped it has been possible to carry on observations of the arable weed flora as it appears in the field in order to make comparisons with what happens under glasshouse conditions.

The most striking feature of the New Zealand lists is the large number of arable seedlings that appeared in the greenhouse compared with those obtained from Geescroft and Barn Field soils, the numbers being 457 and 334 respectively from the two New Zealand samples against 74 from Geescroft and 12 from Barn Field grass. This is easily accounted for by the relatively short time that New Zealand field had been down to grass, as many seeds are known to be able easily to retain their vitality for over 10 years. On the other hand the number of true grassland plants was less than in the other two cases, because the shorter period during which the field was grassed over had not permitted such a large stock of ungerminated, living seeds to accumulate. When all the seedlings exclusive of grasses are considered together, it is seen that in the fields under consideration the total number decreases as the length of time under grass increases. This, however, must not be taken as a definite statement of general application, because so much depends upon the initial state of cultivation of the ground, the store of arable seeds originally in the soil, and the particular soil condition which determines how long the seeds are able to retain their vitality. Probably, however, the statement would approximately hold good under ordinary circumstances provided ample allowance were made for individual and local variations.

Altogether 16 arable species were derived from buried seeds, but 98 % of the total number of seedlings were referable to nine of these species. The number of seedlings of each individual species obtained from each separate hole varied within wide limits, as from 1 to 36 with *Papaver*

TABLE VI. *New Zealand Grass.* Holes 1 N, 2 N, 1 S, 2 S.

		Depth in inches													
		1	2	3	4	5	6	7	8	9	10	11	12	Total for each hole	Grand Total
Arable weeds															
Alchemilla arvensis	Lady's Mantle	.	.	.	.	.	.	1	.	.	.	.	.	0+ 0+ 1+ 0	1
Arenaria serpyllifolia	Thyme-leaved Sandwort	.	.	.	.	1	.	.	.	.	.	.	.	1+ 0+ 0+ 0	1
Atriplex patula	Orache	.	2	8	5	6	7	3	8	9	24	8	26	24+ 6+ 41+ 35	106
Brassica sp.	Charlock	1	1	1	1	3	1	.	2	.	1	.	.	0+ 6+ 3+ 2	11
Euphorbia exigua	Dwarf Spurge	7	7	9	9	7	3	2	2	.	.	.	.	8+ 2+ 5+ 31	46
Lapsana communis	Nipplewort	.	.	.	.	.	1	.	.	.	.	.	.	0+ 0+ 1+ 0	1
Papaver sp.	Poppy sp.	7	11	6	16	16	12	4	2	.	.	.	.	8+43+ 15+ 8	74
Polygonum aviculare	Knot-grass	4	7	7	5	6	7	5	1	4	19	1	.	32+ 1+ 18+ 15	66
Sonchus asper	Corn Sowthistle	7	5	7	11	7	3	.	.	.	.	.	.	29+ 6+ 3+ 2	40
Veronica agrestis	Field Speedwell	12	9	1	7	2	.	1	1	.	.	.	.	3+ 1+ 18+ 11	33
„ hederaefolia	Ivy-leaved Speedwell	3	2	3	6	3	1	1	.	.	.	.	.	2+ 5+ 5+ 7	19
„ Tournefortii	Large Field Speedwell	26	7	15	7	1	1	.	.	.	.	.	.	23+ 6+ 15+ 13	57
„ sp.	Speedwell sp.	.	.	.	.	1	.	.	.	.	.	.	.	1+ 0+ 0+ 0	1
Viola tricolor	Wild Pansy	1	.	.	.	.	.	.	.	.	.	.	.	0+ 1+ 0+ 0	1
	Total	68	51	57	67	53	36	17	16	13	44	9	26	131+77+125+124	467
Arable or grassland plants															
Capsella Bursa-pastoris	Shepherd's Purse	.	.	.	1	1	.	.	.	.	.	.	.	0+ 0+ 0+ 2	2
Cerastium vulgatum	Mouse-ear Chickweed	5	.	.	1	1	.	.	.	.	.	.	.	0+ 0+ 0+ 7	7
Cirsium arvense	Creeping Thistle	.	.	1	1	.	.	.	.	.	.	.	.	1+ 1+ 0+ 0	2
Convolvulus arvensis	Bindweed	.	3	.	.	.	.	.	.	.	.	.	.	3+ 0+ 0+ 0	3
Myosotis arvensis	Field Forget-me-not	.	1	.	.	.	.	.	1	.	.	.	.	1+ 0+ 0+ 1	2
Plantago major	Greater Plantain	6	5	1	1	2	.	.	.	.	.	.	.	2+13+ 0+ 0	15
Ranunculus repens	Creeping Buttercup	.	1	2	.	.	.	.	.	.	.	.	.	0+ 0+ 0+ 3	3
„ sp.	Buttercup sp.	.	.	.	2	.	.	.	.	.	.	.	.	0+ 0+ 0+ 2	2
Rumex sp.	Dock sp.	1	.	.	.	.	.	.	.	.	.	.	.	0+ 0+ 1+ 0	1
Stellaria media	Chickweed	.	2	2	4	2	2	2	.	.	1	.	.	4+ 2+ 5+ 4	15
	Total	12	12	6	10	6	2	2	1	.	1	.	.	11+16+ 6+ 19	52

Grassland plants															
Ajuga reptans	Bugle	1	.	.	.	.	.	.	.	.	.	.	.	0+ 1+ 0+ 0	1
Hypochaeris radicata	Cat's-ear	1	.	.	1	1	.	.	.	.	.	.	.	0+ 1+ 2+ 0	3
Rubus Idaeus	Raspberry	4	.	.	1	.	.	.	.	.	.	.	.	0+ 4+ 0+ 1	5
Trifolium pratense	Red Clover	11	3	6	7	3	.	1	.	.	.	.	.	22+ 8+ 0+ 1	31
" repens	White Clover	53	6	.	.	1	4	.	1	.	.	.	.	32+19+ 10+ 4	65
" " *or* pratense	" " *or* Red Clover	13	.	.	.	.	.	.	.	.	.	.	.	13+ 0+ 0+ 0	13
" sp.	Clover sp.	.	1	.	.	.	.	.	.	.	.	.	.	1+ 0+ 0+ 0	1
	Total	83	10	6	9	5	4	1	1	.	.	.	.	68+33+ 12+ 6	119
														Less Leguminosae	9
Grasses															
Agrostis alba	Bent-grass	2	6	.	5	11	13	5	.	2	.	.	.	14+ 8+ 11+ 11	44
" vulgaris	"	7	2	3	4	1	1	.	2	.	.	.	.	11+ 4+ 4+ 1	20
Dactylis glomerata	Cocksfoot	4	1	.	.	.		.	.	.	.	.	.	4+ 0+ 0+ 1	5
Festuca ovina	Sheep's Fescue	1	.	.	.	.	.	.	.	.	.	.	.	1+ 0+ 0+ 0	1
Lolium perenne	Perennial Rye-grass	30	2	1	6	.	.	1	.	1	.	.	.	4+19+ 14+ 4	41
Grasses, various		2	5	1	3	1	1	1	.	.	.	.	.	8+ 1+ 3+ 2	14
	Total	46	16	5	18	13	15	7	2	3	.	.	.	42+32+ 32+ 19	125
Suspected intruders															
Senecio vulgaris	Groundsel	.	1	1	.	.	.	.	.	.	.	.	.	1+ 0+ 1+ 0	2
Sonchus oleraceus	Common Sowthistle	.	1	.	.	2	.	.	1	.	.	.	2	1+ 1+ 2+ 2	6
	Total	.	2	1	.	2	.	.	1	.	.	.	2	2+ 1+ 3+ 2	8

TABLE VII. *New Zealand Grass.* Holes 1 BN, 2 BN, 1 BS, 2 BS.

		Depth in inches 1	2	3	4	5	6	7	8	9	10	11	12	Total for each hole	Grand Total
Arable weeds															
Aethusa Cynapium?	Fool's Parsley?	.	.	.	.	.	1	.	.	.	.	.	.	0 + 0 + 0 + 1	1
Atriplex patula	Orache	1	4	10	5	9	6	10	11	4	16	12	.	12 + 35 + 26 + 15	88
Brassica sp.	Charlock	.	2	3	.	4	2	.	.	.	.	.	.	1 + 6 + 2 + 2	11
Euphorbia exigua	Dwarf Spurge	2	4	5	4	3	4	1	1	.	.	.	.	7 + 1 + 8 + 8	24
Papaver sp.	Poppy sp.	12	19	7	8	5	3	2	.	.	.	.	.	1 + 12 + 7 + 36	56
Polygonum aviculare	Knot-grass	8	4	10	7	9	8	1	4	3	.	1	.	5 + 13 + 31 + 6	55
Sonchus asper	Corn Sowthistle	.	1	2	3	5	2	.	.	.	.	.	.	2 + 5 + 4 + 2	13
Veronica agrestis	Field Speedwell	17	2	1	2	5	1	.	.	.	.	.	.	4 + 0 + 18 + 6	28
" arvensis	Wall "	1	1	.	1	.	.	.	.	.	.	.	.	0 + 0 + 3 + 0	3
" hederaefolia	Ivy leaved Speedwell	3	1	8	3	4	3	.	.	.	.	.	.	2 + 2 + 10 + 8	22
" Tournefortii	Large Field "	10	5	4	3	3	.	.	.	.	.	.	.	17 + 1 + 3 + 4	25
" sp.	Speedwell sp.	3	3	.	.	.	.	.	.	.	.	.	.	3 + 0 + 1 + 2	6
Viola tricolor	Wild Pansy	.	.	1	.	1	.	.	.	.	.	.	.	0 + 1 + 1 + 0	2
	Total	57	46	51	36	48	30	14	16	7	16	13	.	54 + 76 + 114 + 90	334
Arable or grassland plants															
Capsella Bursa-pastoris	Shepherd's Purse	.	.	.	1	.	.	.	.	.	.	.	.	0 + 0 + 1 + 0	1
Cerastium vulgatum	Mouse-ear Chickweed	3	.	.	.	1	.	.	1	.	.	.	.	0 + 2 + 3 + 0	5
Myosotis arvensis	Field Forget-me-not	4	3	3	.	.	.	.	.	.	.	.	.	3 + 2 + 4 + 1	10
Plantago major	Greater Plantain	1	.	.	1	.	.	.	.	.	.	.	.	0 + 1 + 1 + 0	2
Rumex sp.	Dock sp.	8	2	2	1	1	1	1	1	1	.	.	.	1 + 5 + 12 + 0	18
Scabiosa arvensis	Field Scabious	.	.	.	.	.	.	.	.	.	1	.	.	0 + 0 + 1 + 0	1
Stellaria media	Chickweed	5	1	1	1	3	1	.	.	.	.	.	.	0 + 5 + 0 + 7	12
	Total	21	6	6	4	5	2	1	2	1	1	.	.	4 + 15 + 22 + 8	49
Grassland plants															
Trifolium pratense	Red Clover	20	2	1	1	2	2	1	.	.	.	.	.	10 + 9 + 0 + 10	29
" repens	White "	21	4	1	.	2	.	.	.	.	.	.	.	7 + 6 + 13 + 2	28
	Total	41	6	2	1	4	2	1	.	.	.	.	.	17 + 15 + 13 + 12	57
Grasses															
Agrostis alba	Bent-grass	.	1	3	7	1	3	6	2	.	.	.	.	7 + 11 + 3 + 2	23
" vulgaris et sp.	"	8	2	1	3	.	1	1	.	.	.	.	.	1 + 7 + 5 + 3	16
Dactylis glomerata	Cocksfoot	.	1	.	.	.	.	.	.	.	.	.	.	1 + 0 + 0 + 0	1
Festuca ovina	Sheep's Fescue	.	.	.	.	.	.	.	2	.	.	.	.	1 + 1 + 0 + 0	2
Lolium perenne	Perennial Rye-grass	49	4	1	2	.	.	.	1	.	.	.	.	46 + 5 + 5 + 1	57
Grasses, various		2*	3	.	.	1	5	8	2	.	1	.	.	* + 10 + 1 + 11	22*
	Total	59*	11	5	12	2	9	15	7	.	1	.	.	56* + 34 + 14 + 17	121*
Suspected intruders															
Senecio vulgaris	Groundsel	1	4	.	1	1	.	1	.	.	.	.	1	0 + 4 + 2 + 3	9
Sonchus oleraceus	Common Sowthistle	1	.	1	.	1	.	.	1	.	.	.	.	2 + 1 + 0 + 1	4
	Total	2	4	1	1	2	.	1	1	.	.	.	1	2 + 5 + 2 + 4	13

sp., and from 6 to 41 with *Atriplex patula*, but with two single exceptions in the case of *Veronica agrestis* and *Brassica* sp. each of the main seedlings appeared from every one of the eight sampled holes in greater or less quantity. This shows that these species must have been very abundant in the field at the time of grassing down and also that the distribution was widespread and not localised in particular areas, as the sampled holes were taken from widely separated parts of the field and were not huddled together in one place. *Brassica* was well distributed, but the seedlings were not very plentiful in comparison to the other main species. The remaining less plentiful species all occurred as isolated individuals, indicating that they were either very local in distribution, or else that the seeds were less resistant to burial and had nearly all succumbed during the 10 years under grass. Information on these points was sought from the field itself during the first year after the land was ploughed. Ploughing took place in the autumn of 1915 and the weeds were examined in the following February, when the situation was as follows with regard to arable seedlings:

Veronica Tournefortii „ hederaefolia Brassica sp.	plentiful
Papaver sp., especially plentiful at one end of area	
Veronica, probably agrestis Sonchus asper Senecio vulgaris	occasional
Matricaria inodora Veronica arvensis Scandix Pecten	scarce

In addition to the purely arable weeds the following were in evidence:

Rumex crispus, frequent	
Stellaria media Ranunculus repens	occasional
Senecio Jacobaea Lamium purpureum Chrysanthemum leucanthemum Taraxacum vulgare Capsella Bursa-pastoris Convolvulus arvensis Trifolium repens Centaurea nigra (not seedlings)	scarce

It thus appears that during the winter months immediately succeeding the ploughing four of the species that appeared most plentifully from the seeds buried in the soil samples came up in quantity on the field and two other of the main species were present in less amount. It is impossible

to conceive any way in which great quantities of *Veronica Tournefortii*, *V. hederaefolia* and *Brassica* seeds could have been imported on to New Zealand field during or after the ploughing. In fact, it would have been utterly impossible in the case of *Veronica hederaefolia*, as this plant is an exceedingly early annual, and by the end of June or earlier it has shed its seed *in situ* and the plants have died down. The seeds are produced in abundance but have no provision for carriage by wind or animals, so that after shedding they become mixed in with the soil where they fall and lie dormant till the next season. As New Zealand was under grass till the autumn of 1915, and seedlings of *V. hederaefolia* were plentiful by February, 1916, the proof is conclusive that the plants arose from seeds which had lain dormant in the soil since the grassing down in 1906.

Although six of the nine main species had appeared by February, 1916, no trace was observed of the most abundant of all, *Atriplex patula*, nor of *Euphorbia exigua* nor of *Polygonum aviculare*. This, however, accords absolutely with expectations and with the glasshouse tests. In the latter experiments two or three seeds of each of the three species germinated soon after the pans were set up, in September and early October, after which no more seedlings appeared till the following March or April, when germination occurred freely. Evidently the winter period is inimical to germination in these cases, and consequently the field was bare of the seedlings in February, although by the following August they were all present in abundance. The species that were present on the field in February kept up a constant succession of germination in the pans throughout the winter, so that in this respect also the field and glasshouse observations correspond closely. When the field was examined later in 1916, all the arable species were found that had appeared in the pans, with the single exception of *Arenaria serpyllifolia*. This was apparently very scarce, and if plants existed on the field they may have been overlooked or confused with small plants of *Stellaria media*. The dominance of charlock was very marked, but it may be suggested that this was not necessarily due to the very large number of plants occurring per unit area, but to the large size that individuals attain and to the showiness of the flowers. This explanation may help to reconcile the disparity between the dominance in the field and the comparative fewness of the seedlings raised in the greenhouse.

The number of viable arable seeds obtained from the New Zealand soil samples is in reality enormous and represents about 17 million per acre, calculated on the average of the samples taken. Such calculations are not really worth much, but they serve to show that even after land has

been grassed over for 10 years vast numbers of arable seeds retain their vitality and are ready to spring up and dominate the situation at the first favourable opportunity. When once the arable weeds have their opportunity the true grassland plants have little chance and make a very poor show a few months after ploughing.

C. ARABLE LAND.

(1) *Long Hoos.*

This field has been and is under ordinary farm management, so that the weed seeds that germinated during the experiment had not been influenced in number or condition by any period during which the land had been under grass. Consequently the results indicate the normal state of affairs in land which is cultivated under rotation cropping in which experimental methods have played no part. The number of seedlings obtained was enormous—although only two holes were sampled, representing an area of ½ sq. foot, no less than 782 arable weed seeds, representing over 68 millions per acre, germinated during the sixteen months that the experiment had been carried on. Up to the previous December (1916) 524 seedlings had appeared, so that an additional 258 seeds started into growth during the second season. These numbers are very large, being four times as many as were obtained from a similar area over a longer period from New Zealand field, which had been under grass for 10 years. The amount of possible competitive damage that can be done by weeds in the absence of efficient cultivation is strongly emphasized by these results, as if the weeds were left undisturbed to germinate and fight their own mutual battle, the introduced crop plants would have little chance in the fray.

The number of arable weed species that appeared were greater than in any other set of samples that were taken. *Arenaria serpyllifolia*, *Alchemilla arvensis*, *Veronica Tournefortii*, and *Matricaria inodora* were the most abundant, contributing altogether 70 % of the total, so that they are evidently very widely distributed over the field. *Anagallis arvensis*, *Galium* sp., *Polygonum Convolvulus*, *Sonchus asper* and *Caucalis* sp. were very scarce, indicating that these species are more or less local and occasional in their distribution. It is noticeable that though *Atriplex patula* is one of the weeds that is most resistant to long burial on this soil, yet it is not one of the most abundant species, and apparently its distribution is somewhat irregular, as the numbers obtained from the two holes vary so widely.

TABLE VIII. *Long Hoos.* Holes 1, 2.

		Depth in inches												Total for each hole	Grand Total
		1	2	3	4	5	6	7	8	9	10	11	12		
Arable weeds															
Alchemilla arvensis	Lady's Mantle	9	14	21	21	13	15	19	11	5	.	.	.	60 + 68	128
Anagallis arvensis	Poor Man's Weather-glass	.	.	.	.	.	.	.	1	.	.	.	.	0 + 1	1
Arenaria serpyllifolia	Thyme-leaved Sandwort	22	34	52	16	44	14	15	9	4	1	1	.	158 + 54	212
Atriplex patula	Orache	1	3	2	3	4	1	4	3	.	8	1	.	30 + 0	30
,, ,, *or* Chenopodium	Orache or Fat Hen	.	.	.	.	.	.	2	.	.	17	1	3	20 + 3	23
Brassica sp.	Charlock	.	3	1	1	3	2	.	2	1	1	2	.	8 + 8	16
Caucalis sp.		.	.	.	.	.	.	.	.	1	.	.	1	2 + 0	2
Euphorbia exigua	Dwarf Spurge	1	1	.	.	1	5	1	1	2	.	.	.	6 + 6	12
Galium (Aparine *or* tricorne)	Bedstraw, Cleavers	.	.	.	.	.	.	.	.	.	.	.	1	1 + 0	1
Matricaria inodora	Scentless Mayweed	10	13	26	9	13	10	6	1	.	.	1	6	46 + 49	95
Papaver sp.	Poppy sp.	6	5	16	10	8	3	2	2	.	1	.	.	21 + 32	53
Polygonum aviculare	Knot-grass	.	1	5	2	1	8	2	2	1	1	.	.	9 + 14	23
,, Convolvulus	Black Bindweed	.	.	.	.	.	.	.	1	1	.	.	.	1 + 1	2
,, Persicaria	Willowweed	.	.	.	.	.	2	.	2	.	.	.	.	3 + 1	4
Sonchus asper	Corn Sowthistle	.	.	.	1	.	.	.	.	.	.	.	.	0 + 1	1
Veronica agrestis	Field Speedwell	3	5	2	1	4	5	.	.	.	.	.	.	10 + 10	20
,, hederaefolia	Ivy-leaved Speedwell	.	1	1	.	.	.	.	.	.	.	.	1	3 + 0	3
,, Tournefortii	Large Field Speedwell	4	9	25	32	24	3	8	.	.	.	.	.	79 + 26	105
,, sp.	Speedwell sp.	3	.	3	.	.	9	2	4	3	.	.	.	11 + 13	24
Viola tricolor	Wild Pansy	1	3	8	1	.	5	2	1	3	.	2	1	20 + 7	27
	Total	60	92	162	97	115	82	63	40	21	29	8	13	488 + 294	782
Arable or grassland plants															
Cerastium vulgatum	Mouse-ear Chickweed	3	2	4	2	.	1	.	2	.	.	.	.	2 + 12	14
Myosotis arvensis	Field Forget-me-not	2	.	3	.	1	1	2	.	.	.	.	.	0 + 9	9
Plantago major	Greater Plantain	2	.	.	2	1	1	1	.	.	.	.	.	4 + 3	7
Ranunculus sp.	Buttercup sp.	1	.	.	.	.	.	.	.	.	.	.	.	0 + 1	1
Scabiosa arvensis	Field Scabious	.	.	.	.	.	.	.	1	.	.	.	.	1 + 0	1
Stellaria media	Chickweed	.	.	1	.	.	.	.	2	.	.	.	.	3 + 0	3
	Total	8	2	8	4	2	3	3	5	.	.	.	.	10 + 25	35
Grassland plant, probably left from previous crop															
Trifolium pratense	Red Clover	.	.	.	.	.	.	.	.	1	.	.	1	2 + 0	2
Grasses															
Agrostis sp.	Bentgrass	3	5	7	3	3	4	6	3	5	1	2	1	22 + 21	43
Poa annua	Annual Meadow-grass	2	1	.	2	.	3	.	.	.	.	.	.	3 + 5	8
Grasses, various		.	.	.	2	1	2	1	.	.	.	.	.	6 + 0	6
	Total	5	6	7	7	4	9	7	3	5	1	2	1	31 + 26	57
Suspected intruders															
Senecio vulgaris	Groundsel	1	.	.	1	2	1	.	1	.	1	.	.	4 + 3	7
Sonchus oleraceus	Common Sowthistle	.	.	2	1	.	.	.	.	.	.	.	.	2 + 1	3
	Total	1	.	2	2	2	1	.	1	.	1	.	.	6 + 4	10

A certain number of weeds appeared that are common to arable and grass land, but they are evidently present here in their character of arable weeds. The true grassland plants were represented by one solitary species, *Trifolium pratense*, of which only two plants appeared. Few grasses occurred except for a number of *Agrostis* sp. which is a typical arable weed and should really be included in the list of arable seedlings. raising the grand total to 782 + 43 = 825.

(2) *Barn Field* (8·0).

Barn Field has carried root crops year after year for 61 years since 1856, and the plot 8·0 has received no manure of any kind during the whole period. As a result of this treatment the soil is now much impoverished and supports only a feeble type of vegetation, and in addition the continual hoeing and cultivating has reduced the weeds to a minimum, as few of them have any opportunity of forming seed before they are hoed up. This is well shown by the results obtained from the soil samples. Two holes were sampled, making up 24 pans. During the whole 16 months of the experiments only eight pans produced any seedlings, and three out of the eight were occupied solely by *Senecio vulgaris*, a possible intruder. *Senecio vulgaris* occurs rather frequently on Barn Field, and as most of the seedlings appeared in the pans during the first three months of the experiment it is quite likely that the seeds were really associated with the soil and were introduced with it, so that in this case they would not be intruders. Altogether seven arable seedlings and three arable or grassland plants appeared, the grand total of 20 seedlings from an area of ½ sq. foot of soil being made up by 10 *Senecio vulgaris* seedlings. Every seedling appeared within the top six inches of soil, and furthermore, all the seedlings made very feeble growth, showing that the seeds were weak. The paucity in the number of species of arable weed seeds is probably more due to the continual cultivation for roots than to the starved condition of the soil, as even on the heavily manured plots in the same field only nine or 10 species of weeds occur, and these are present in very small quantity.

(3) *Agdell Field* (5).

This plot has been left unmanured since 1848 and has been worked under four course rotation experiments during the period of 69 years. In the third year of each course the plot has been left fallow, so that specially good opportunities have arisen of cleaning the land, as the seedlings that germinate during the fallow time are to a large extent

TABLE IX. *Barn Field Arable* (8·0). Holes 1, 2.

		Depth in inches 1	2	3	4	5	6	7	8	9	10	11	12	Total for each hole	Grand Total
Arable weeds															
Euphorbia peplus	Petty Spurge	.	1	2	.	.	1	.	.	.	.	.	.	0+4	4
Sonchus asper	Corn Sowthistle	1	.	.	.	.	1	.	.	.	.	.	.	0+2	2
Veronica Tournefortii	Large Field Speedwell	.	.	.	.	.	1	.	.	.	.	.	.	1+0	1
	Total	1	1	2	.	.	3	.	.	.	.	.	.	1+6	7
Arable or Grassland plants															
Centaurea nigra	Hardhead	.	.	1	.	.	.	.	.	.	.	.	.	0+1	1
Stellaria media	Chickweed	.	.	1	.	.	.	.	.	.	.	.	.	0+1	1
	Total	.	.	2	.	.	.	.	.	.	.	.	.	0+2	2
Possible intruders															
Senecio vulgaris	Groundsel	7	2	.	.	1	.	.	.	.	.	.	.	5+5	10
Sonchus oleraceus	Common Sowthistle	1	.	.	.	.	.	.	.	.	.	.	.	0+1	1
	Total	8	2	.	.	1	.	.	.	.	.	.	.	5+6	11

TABLE X. *Agdell* (5). Holes 1, 2.

		Depth in inches 1	2	3	4	5	6	7	8	9	10	11	12	Total for each hole	Grand Total
Arable weeds															
Alchemilla arvensis	Lady's Mantle	.	.	1	.	.	.	.	.	.	1	.	.	1+ 1	2
Arenaria serpyllifolia	Thyme-leaved Sandwort	.	.	.	.	.	.	1	7	1	.	.	.	0+ 9	9
Atriplex patula	Orache	4	5	.	.	.	2	1	.	.	.	.	.	1+11	12
,, ,, *or* Chenopodium	,, *or* Fat Hen	.	.	.	2	2	.	3	.	.	.	.	.	0+ 7	7
Chenopodium album	Fat Hen	.	.	4	.	1	.	.	.	.	.	.	.	0+ 5	5
Euphorbia exigua	Dwarf Spurge	.	.	.	.	.	.	1	.	.	.	.	.	1+ 0	1
Galium sp.	Bedstraw sp.	.	.	.	1	.	.	.	.	.	.	.	.	1+ 0	1
Matricaria inodora	Scentless Mayweed	1	.	.	1	1	1	1	.	.	.	.	.	2+ 3	5
Polygonum aviculare	Knot-grass	.	.	.	.	1	.	.	.	.	.	.	.	1+ 0	1
	Total	5	5	5	4	5	3	7	7	1	1	.	.	7+36	43
Grassland plants															
Trifolium pratense	Red Clover	14	6	23	18	15	33	17	13	.	1	.	.	53+87	140
,, repens	White Clover	1	.	1	2	.	3	13	3	.	.	.	.	16+ 7	23
,, (cut-leaved)	Clover sp.	.	.	.	1	.	2	1	.	.	.	.	.	1+ 3	4
	Total	15	6	24	21	15	38	31	16	.	1	.	.	70+97	167
Died without developing	Total	3	6	.	17	5	4	.	4	.	.	.	.	24+15	39
Suspected intruder															
Senecio vulgaris	Groundsel . Total	.	.	1	.	.	.	.	.	.	.	.	.	0+ 1	1

cultivated out and prevented from seeding. Also, as the field is experimental, an extra amount of weeding is done during the growing seasons, and this also tends to reduce the quantity of weeds. Added to this, the soil is now very poor owing to the long continued cropping without manure, and this impoverishment influences the growth of weeds as well as of crops and tends to reduce their vigour and abundance. All these limiting factors are reflected in the number of buried weed seeds which germinated in the soil samples.

From the two holes covering ½ sq. foot only 43 arable weeds were identified, though probably some of the 39 seedlings which died in infancy without developing belonged to the same category. These numbers are in sharp contrast to those obtained from Long Hoos where no experimental interference took place and where the land received manure at intervals in the ordinary course of management.

During the first few months to Dec. 1916, *Atriplex patula* and *Chenopodium album* were the only weed seeds that germinated, and few even of these were present. In the spring of 1917 the number of these species increased considerably, and various other plants came up, bringing the total arable species to eight. Several plants of *Arenaria serpyllifolia* and *Matricaria inodora* appeared, but the other species only occurred in ones or twos. *Trifolium pratense* was present in far greater quantity than the weed seeds, but this is probably the result of an accidental error, as a few years ago clover seed was sown on the fallow half instead of on the clover half of the field, and it has not yet been possible to entirely eliminate the species. Both *Trifolium pratense* and *T. repens* were present in quantity from six to eight inches below the surface, and consequently it is probable that many of the seedlings were derived from seeds that had been buried for some considerable time and had been worked down by ploughing and cultivation.

GENERAL DISCUSSION OF THE RESULTS.

A survey of Tables I–X shows at a glance how closely the flora derived from buried seeds is associated with the history of the land. Permanent grassland is practically devoid of arable weeds, and also the number and variety of the species that do occur is greatly influenced by the fact of grazing or cutting, as the case may be. Continual close grazing, as on the Common, hinders seed production, and so reduces the number of viable seeds that become buried in the soil. Continual mowing, as on the

Park Grass, allows of the ripening of the seeds of many species in a differential degree, the earlier species having a better chance than those which flower later in the season. Consequently far more seeds become buried in this case, and the flora tends to remain very varied.

A striking difference exists between the buried seed flora of permanent grassland and of land that has at one time been under the plough, even though nearly 60 years have elapsed since grassing down. The permanent grassland is largely colonised by species of grasses and miscellaneous plants which are definitely associated with pasture and never with arable land, except for the one or two arable weeds which must be of accidental origin. On land that was originally arable however, a large number of plants occur, such as hardhead, mouse-ear chickweed, ribwort plantain, chickweed, etc., which are common to both arable and grassland, indicating that when once these species are established on an area they can persist, even when the type of cultivation is changed.

A fair number of true arable weeds appear even from soil that has been grassed over for 58 years (Laboratory House Meadow) and, as was shown in the special discussion above, many of these may almost certainly be regarded as survivors from seeds left in the soil from the time of arable cultivation. The proportion of grassland plants is large compared to that of the arable weeds, a result that is in complete accordance with expectations. Geescroft field has been under grass for a shorter period of time, and the number of arable seeds is greater, while the proportion of grassland plants has decreased. This trend of events becomes more marked as the time of grassing down gets less, and on New Zealand field, with only 10 years under grass, the arable weeds bear a heavy proportion to the grassland plants, particularly if the clovers (which might have been derived from buried seeds of a sown crop) are left out of consideration. The transition from the state of affairs on such temporary grassland to that on ordinary ploughed land is gradual and in the same sense, the number of arable weeds being greatly increased, the arable or grassland plants being sparsely represented, while the true grassland plants are almost absent.

The changes in the proportion of the arable and grassland plants derived from buried seeds are so consistent and so regularly associated with the history of the land that one is irresistibly forced to the conclusion that when arable land is grassed over a certain number of the seeds are able to retain their vitality for very many years. Many of the seeds die within a comparatively short time after burial, and as time goes on the number of living seeds gradually becomes less, though the evidence goes

to show that some seeds will survive burial for at least 58 years. Usually most of the older arable seeds survive in the lower depths of soil where the conditions are less variable, whereas the shorter the time that land has been under grass the greater the proportion of arable seeds that are found near the surface. While the stock of arable seeds is diminishing with the lapse of time, the supply of grassland seeds is being augmented by the fresh seeds that are ripened by the surface vegetation and are gradually carried down into the soil. Naturally enough, the greater number of these seeds are found in the upper inches of soil, comparatively few penetrating below the eighth inch.

Such fields as Barn Field Arable and Agdell show clearly how close is the connection between the methods of cultivation and the supply of weed seeds that become buried in the soil. Continual cultivation for roots, as on Barn Field, keeps most of the weeds from seeding and so prevents the accumulation of large stores of seeds in the soil. On the Agdell plot it is probable that many of the weeds have become starved out, or so impoverished that their seeds are not strong enough to survive for any length of time. Neither of these instances, however, is at all normal, as they are the result of experiment and not of ordinary farm management.

From consideration of the results of the above experiments it may be concluded that the case for large crops of charlock, poppies or other arable weeds appearing when real old pasture is ploughed up must still be regarded as not proven. These particular weeds were not obtained in the soil from old pastures, nor were they present when the land had been under grass for nearly sixty years. Nevertheless it is evident that under suitable conditions the seeds of some weeds are able to lie dormant at various depths in the soil over long periods, and to start into activity if and when the method of cultivation is so changed that they are brought nearer to the surface into the presence of the right combination of warmth, air and moisture. Consequently, the large crops of weeds that appear when temporary pasture is ploughed up must be regarded as being derived from seeds buried in the soil, and not from seeds transported from other areas by external agencies.

(*Received October 8th*, 1917.)

THE INFLUENCE OF POTSHERDS ON NITRIFICATION IN THE INDIAN ALLUVIUM.

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(With Four Figures in text.)

INTRODUCTION.

NITROGEN, one of the most important plant nutrients, is present in the soil mainly as organic compounds which, with certain exceptions, are not, in those forms, assimilable by ordinary cultivated plants. The organic compounds undergo a series of chemical changes before they are ultimately changed into nitrates which are practically the most useful compounds of nitrogen which can be taken up by plants.

Decomposition of organic materials, however, depends on many factors, one of the most potent of which is the supply of air, as oxygen gas is largely used up in some of the reactions involved. Hence the freer the aeration of the soil, the better are the decay and nitrification of the organic matter. Moreover the greater the oxygen available, the more active are the fungi of the soil which collect phosphates and potash for the nourishment of higher plants. It thus follows that the more efficient the soil aeration, the greater is the amount of plant food available. Mr A. Howard, C.I.E., Imperial Economic Botanist, has described the important rôle that potsherds play as aerating agents and has drawn attention to the various factors determining their practical value in effecting improvements in soils[1]. Not only do potsherds bring about beneficial bio-chemical effects, but they also improve the physical condition of the soil and favour an abundant and rapid development of roots of plants and thus tend to increase the yield of crops.

[1] "Soil Ventilation and Soil Aeration in Agriculture," *Agr. Res. Inst* Pusa, Bull. 52 and 61; also "The Manurial Value of Potsherds," *Agric. Journ. India*, 1916, **11**, p. 256.

In this connection, Mr Howard had quoted the analysis of a sample of well water at Jais in the district of Rae Bareli, where rich crops of good quality tobacco are grown with the help of irrigation water from wells. The water was rich in nitrates and potash and the amount of dissolved oxygen was high. In all these respects the sample was markedly superior to the well waters at Pusa. Mr Howard has pointed out that the Jais wells are situated in land where potsherds exist in abundance and where the aeration of the soil is copious. At Pusa, on the other hand, the alluvium is fine and soil aeration is difficult. Here the well waters do not possess any great manurial value, whereas the Jais waters are much valued for irrigating tobacco.

With a view of finding out the extent to which nitrification is influenced by the presence of potsherds in Pusa soil, some preliminary experiments have been undertaken.

As the nitrification of organic materials may be accepted as a fair criterion of the availability of the nitrogen contained in them, instead of examining the complete nitrogen cycle, only the formation of nitrates —the final oxidation products—has been studied.

EXPERIMENTS IN JARS.

In this set of experiments, manured soil mixed with different amounts of potsherds was used and the drainage waters from these were examined. Farm yard manure, containing 68·22 per cent. moisture and 0·47 per cent. nitrogen, was applied at the rate of three pounds per cubic foot. The following proportions of potsherds were used: nil, ten per cent., twenty per cent., and thirty per cent. of the total volume.

Stoneware cultivation jars, provided with drainage holes at the bottom, were filled with the soil mixtures. First, a two inch layer of *bajri* (small, roundish, hard pieces of bricks from ⅛ to ¼ inch in diameter) was placed at the bottom of each jar to serve as a percolation layer. The proper soil mixture was then put in; each handful, as it was introduced, being levelled and uniformly pressed in. Attempt was made to pack the soil in an uniform manner in all the jars and to have it as nearly compact as in the field.

There were altogether twelve jars, consisting of three groups of four jars each. They were filled up with 0 per cent., ten per cent., twenty per cent. and thirty per cent. potsherds respectively. In the first group, the soil layer was eight inches deep, in the second it was twelve inches and in the third, twenty inches.

The jars were kept almost wholly embedded in the earth and the soils therein were practically exposed to the same temperature conditions as the soil in the field. Arrangements were provided to prevent the access of rain water into the bottles which collected the percolates. At first, the jars were periodically treated with measured volumes of water. Later on, this method of artificial irrigation was discontinued. Rain was allowed to fall on the jars, and the resulting natural percolates examined.

The jars were filled up on the 7th May. They were irrigated once with well water on the 15th May and the resulting percolates analysed. The following results were obtained.

TABLE 1.

Percolates from the Jars, 15th May, 1916.

Jar No	Internal diameter of jars (in inches)	Depth of soil (in inches)	Per cent potsherds	Well water* added		Percolate			Nitrogen as nitrates		Gain of nitrogen as nitrates (mgms)
				Volume (in litres)	Nitrogen as nitrates (mgms.)	Volume (in litres)	Colour	Dissolved oxygen (parts per million)	Parts per million	Mgms. in total percolate	
1	9	8	0	6·0	31·7	2·13	Brown	4·38	63·0	134·2	102·5
2	9	8	10	6·0	31·7	2·56	Nil	5·85	68·6	175·6	143·9
3	9	8	20	6·0	31·7	2·35	,,	5·74	63·0	148·1	116·4
4	9	8	30	6·0	31·7	2·00	,,	5·11	115·0	230·0	198·3
5	12	12	0	12·0	63·5	3·40	Brown	2·09	112·5	382·5	319·0
6	12	12	10	12·0	63·5	3·30	Nil	5·99	96·7	319·1	255·6
7	12	12	20	12·0	63·5	4·00	,,	6·16	94·5	378·0	314·5
8	12	12	30	12·0	63·5	3·70	,,	4·42	166·6	616·4	552·9
9	12	20	0	16·0	84·6	2·00	Brown	2·92	144·0	288·0	203·4
10	12	20	10	16·0	84·6	4·00	Nil	5·99	121·5	486·0	401·4
11	12	20	20	16·0	84·6	3·10	,,	5·74	139·5	432·5	347·9
12	12	20	30	16·0	84·6	2·50	,,	5·74	216·0	540·0	455·4

* The well water contained 5·99 parts dissolved oxygen and 5·29 parts nitrogen as nitrates per million.

The influence of potsherds was manifest in two ways. Comparing jars filled with the same depth of soil mixture it was seen that in every case the concentration, as well as the total amount of nitrates, was greater in the case of percolates from the jars which contained thirty per cent. of potsherds in the soil. The figures about the dissolved oxygen were also interesting. The presence of potsherds accelerated the oxidation of organic matter. The percolates from jars containing no added potsherds were coloured and low in dissolved oxygen. That these were richer in dissolved organic matter as compared with the clear percolates from other jars was confirmed by determining the amounts of potassium per-

manganate required to oxidise these. The percolates from jars Nos. 1, 5 and 9 (all containing 0 per cent. potsherds) required, per litre, 0·096, 0·094 and 0·069 gram. respectively of oxygen as compared with 0·014 gram. required in the case of the percolate from jar No. 7 (containing twenty per cent. potsherds)[1].

A fortnight later the jars were again irrigated, this time with distilled water. Analyses of the resulting percolates are recorded below.

TABLE II.

Percolates from the Jars, 29*th May*, 1916.

Jar No.	Internal diameter of jar (in inches)	Depth of soil (in inches)	Per cent potsherds	Distilled water* added (litres)	Percolate				
					Volume (in litres)	Colour	Dissolved oxygen (parts per million)	Nitrogen as nitrates	
								Parts per million	Mgms. in total percolate
1	9	8	0	3·0	0·75	Brown	5·40	43·9	32·9
2	9	8	10	3·0	0·67	Nil	6·68	49·5	33·2
3	9	8	20	3·0	0·61	,,	6·73	47·3	28·9
4	9	8	30	3·0	0·64	,,	6·64	49·5	31·7
5	12	12	0	6·0	0·50	Brown	5·84	58·5	29·3
6	12	12	10	6·0	0·80	Nil	6·68	60·8	48·6
7	12	12	20	6·0	0·69	,,	6·73	56·3	38·8
8		12	30	7·0	0·76	,,	6·50	65·2	49·6
9		20	0	9·0	1·20	Brown	5·25	76·5	91·8
10		20	10	9·0	2·40	Nil	6·13	54·0	129·6
11		20	20	9·0	1·15	,,	6·59	58·5	67·3
12		20	30	8·0	0·58	,,	6·36	157·5	91·4

* The distilled water contained 6·64 parts dissolved oxygen per million.

The result of the analysis showed a marked difference in the nature of the percolates from jars containing no potsherds as compared with those from jars containing added potsherds. The percolates from jars without any potsherds in them were coloured and contained less dissolved oxygen than the percolates from the other jars; the difference in the oxygen content was, however, not so marked as in the first set of percolates. At this stage of the experiment artificial irrigation was discontinued and the jars were exposed to the rain and the resulting percolates were examined. Later on the jars were once more irrigated with distilled water in December.

As the monsoon proceeded, the earth-filling round the jars often gave way dislocating and occasionally breaking the tubes through which the

[1] Nitrates were determined colorimetrically by the phenol disulphonic acid method The dissolved oxygen and the oxygen required were estimated by Rideal's and by Forschammer's processes respectively.

percolates drained into the collecting bottles. The necessary repairs were made as speedily as possible; but still there were many breaks in the percolation record. Later on, the glass tubes were replaced by metal tubes, paraffined inside.

Fortunately, the set of jars Nos. 9 to 12 escaped the accidents referred to above. Complete analytical records for the percolates from these jars are noted below.

TABLE III.

Percolates from the Jars Nos. 9—12.

Jar No	Depth of soil mixture (in inches)	Per cent. potsherds	Milligrams Nitrogen as Nitrates in Percolates								
			15/5/16	29/5	2/7	9/7	11/7	13/7	20/7	24/7	26/7
9	20	0	203·4	91·8	—	—	22·3	16·6	12·7	—	4·3
10	20	10	401·4	129·6	54·0	29·5	20·3	18·0	23·2	7·0	11·7
11	20	20	347·9	67·3	51·5	32·2	22·3	—	27·6	4·2	9·3
12	20	30	455·4	91·4	22·8	34·5	96·7	15·9	61·3	—	—
			28/7	8/8	12/8	4/9	14/9	18/9	23/9	11/10	8/12
9	20	0	4·3	53·5	—	52·6	10·6	29·0	2·7	10·3	25·4
10	20	10	9·1	9·8	9·7	28·3	5·6	6·6	2·2	8·7	33·2
11	20	20	11·0	19·3	3·9	40·7	5·6	4·7	0·8	4·1	24·3
12	20	30	53·6	36·9	81·3	66·2	17·9	12·2	8·7	9·5	68·6

During the whole period the following amounts of nitrogen (as nitrates) were washed out:

Jar No.	Per cent potsherds	Mgm. Nitrogen
9	0	539·5
10	10	807·9
11	20	676·7
12	30	1132·9

These results show that potsherds have produced increased nitrification and that thirty per cent. potsherds are specially efficient, the amount of nitrate from the jar filled with this being more than twice the amount washed out from the soil containing no added potsherds. Apparently, the presence of potsherds renders the soil more open and favours gaseous interchange between the soil and the atmosphere. More oxygen is thus available for the nitrification of the organic matter.

The amount of dissolved oxygen was practically the same in all these later percolates, and so, its determination was stopped.

Both the observations as to the marked influence of the presence of thirty per cent. potsherds and as to the constancy of the amount of dissolved oxygen in the later percolates were also found to hold good in the cases of the drainage waters obtained from the other jars (Nos. 1 to 8).

EXPERIMENTS IN LYSIMETERS.

Nitrates resulting from nitrogenous organic materials dissolve in the water present in the soil. The soil solution, however, does not remain stationary but undergoes continual movements. It therefore follows that the efficacy of a nitrogenous manure will not depend merely on the rate of formation of nitrates but will be considerably modified by the various factors which regulate the movements of the soil solution. For example, in the case of two fields where the rates of the formation of nitrates are the same, if the downward movement of the soil solution (drainage) is more rapid in one field than in the other, the nitrates will be more easily leached out from the first field and will thus be less available for the nutrition of plants.

In the experiments with the jars mentioned above the soil was subjected to abnormal conditions. For example, the ordinary movements of the soil solution going on in the field could not occur in these jars, as the soils in these were quite isolated and were not in contact with the earth. In order to study the variation in the nitrate contents of soils (containing potsherds) under ordinary field conditions, the following experiments were simultaneously carried out.

A series of nine pits, each 1/1000 of an acre in superficial area, were dug out in a cultivated field. These had vertical soil walls. The bottom of each pit was carefully sloped towards a point in one side where a bent iron pipe was put in. On this side a brick wall was built up, suitably strengthened by short lengths of flanking walls. For the purposes of demarking the plots and holding irrigation water, each pit was bounded on the surface by small brick walls. These walls were six inches higher than the existing level of the soil and only six inches deep in the top soil.

The pits were first filled with alternate layers of broken bricks and rubbish, to a depth of eight inches. This layer was levelled in the same way as the bottom of the pit. Over this was put a mixture of manured soil and potsherds of the same composition as used in the jars, i.e. ten, twenty and thirty per cent. potsherds.

The soil mixture was put into the pits, layer by layer, about five inches being put at a time and gently pressed with feet before the next layer was put in.

The depths of the soil mixtures in the pits were two feet, three feet and four feet respectively. The mouths of the iron pipes where they entered the pits were suitably protected by pieces of wire gauze and

layers of *bajri*. This arrangement served to prevent the pipe from being blocked up with soil. Any drainage waters which collected at the mouth of the pipe could therefore flow out easily. It is thus seen that the pits are somewhat analogous to the gauges which are often built in connection with the study of drainage waters.

After the soil mixture had been filled in, the gauges were flooded till percolates came out through the iron pipes at the bottom. This application of water produced a settling down of the soil to about the same condition as would happen in the fields.

Samples of soil were taken once every fortnight with the help of boring cylinders and analysed for nitrates.

The results of the analyses are recorded in a tabular statement below.

Table IV.

Nitrogen as Nitrates in the Soils of the Gauges.

Gauge No	Depth in feet	Per cent. potsherds	Nitrogen (as nitrates) Parts per million of dry soil								
			6/7/16	17/7	31/7	14/8	28/8	14/9	28/9	16/10	30/10
I	2	10	1·7	1·3	1·1	0·5	1·4	0·5	2·0	1·1	2·6
II	2	20	3·0	1·0	1·2	2·0	2·1	2·2	1·5	1·9	3·0
III	2	30	6·2	7·5	7·2	1·2	4·2	0·9	1·8	1·8	5·0
IV	3	10	1·2	1·9	1·7	2·3	2·4	1·8	1·4	1·1	3·1
V	3	20	2·5	3·0	2·1	3·5	·9	3·6	1·7	1·3	4·3
VI	3	30	3·4	3·3	2·8	4·5	·7	5·5	2·4	2·5	9·0
VII	4	10	1·6	1·3	0·9	3·7	·0	2·0	0·4	1·4	2·1
VIII	4	20	4·0	1·9	2·4	4·2	·3	2·9	0·4	1 5	1·6
IX	4	30	4·2	3·1	1·9	5·6	·6	5·2	3·6	6·6	6·0
			13/11	27/11	11/12	25/12	8/1/17	22/1	12/2	26/2	
I	2	10	3·9	4·3	6·3	3·5	5·4	7·5	6·9	9·0	
II	2	20	4·4	4·8	9·5	6·3	11·8	10·6	16·8	14·1	
III	2	30	5·1	4·8	7·2	5·0	8·8	7·5	12·3	13·5	
IV	3	10	3·3	3·6	4·1	4·6	5·7	7·8	8·9	11·9	
V	3	20	4·8	3·7	7·7	3·7	8·8	7·5	10·8	11·3	
VI	3	30	5·6	5·6	9·9	9·4	15·6	8·4	13·1	14·2	
VII	4	10	2·6	4·2	12·9	3·3	7·5	5·6	7·9	7·9	
VIII	4	20	3·7	4·7	4·8	3·0	8·6	6·2	11·8	6·4	
IX	4	30	6·4	5·9	18·6	7·2	12·8	10·6	20·6	12·7	

The results of these analyses are entered graphically in the accompanying charts (Nos. 1, 2 and 3). It will be noticed that, except in the case of the 2 ft. gauges during the months of December and onwards, the soils of all the gauges containing thirty per cent. potsherds contain the highest amounts of nitrates as compared with other gauges of the same depth. Even in the case of 2 ft. gauges, although the one containing twenty per cent. potsherds takes the lead during later periods, the concentration of nitrates in the earlier stages was greatest in the gauge con-

taining thirty per cent potsherds. Thus it is seen that although the soils of the thirty per cent gauges were more open and thus liable to a quicker drainage of the nitrates dissolved in the soil solution, they were still generally richer in nitrates than the other soils.

Taking individual gauges, it is seen that the amounts of nitrates were practically the same during the first three months or so. It was only after this period that they suffered an increase. This bears out the utility of applying manures at least three months in advance. In actual practice this has also been found to be the case. For example, in the case of tobacco, the manure should be applied in June or July, as complete nitrification takes time and various intermediate oxidations have first to take place.

The general increase in the content of nitrates has been maintained from October, with occasional fluctuations, till the end of the period of the experiments (February). This increase seems to be due to a combination of favourable causes (see Chart No. 4).

An examination of the rainfall data of this season shows that there were heavy rainfalls in September and few rainless days till the middle of October. From this time there was practically no rainfall except light showers in the latter part of the month and then again towards the end of January and the beginning of February.

A fall of rain greatly modifies the nitrate contents of soils. Not only does it leach out some of the nitrates but is liable to give rise to denitrification. But the advent of rain-water, charged with dissolved oxygen, replenishes the supply of oxygen in the soil atmosphere[1]. Again, after the rain there occurs a downward movement of the excess of water which causes a flow of air in the soil. Hence, after the stoppage of rains, not only do the chances of loss of nitrates by leakage and denitrification diminish but there is set up a stimulus to increased formation of nitrates.

A similar indraught of air takes place when the level of the subsoil water falls lower. In the chart are plotted the levels of the river Gandak on the different dates. The gauges are situated about 400 yards away from the river. The movements of the underground water near the gauges no doubt suffer a "lag" as compared with the rise and fall of the river level, still the curve in the chart may be taken to give a general indication of the nature of the movements of the soil water level.

[1] E. J. Russell and A. Appleyard, "The Atmosphere of the Soil," *Journ. Agric. Sci.* 1915, **7**, p. 1; E. H. Richards, "Dissolved Oxygen in Rain-water," *ibid.* 1917, **8**, p. 331; also E. J. Russell and A. Appleyard, "The Influence of Soil Conditions on the Decomposition of Organic Matter in the Soil," *ibid.* p. 385.

The river rose up to the end of July. After this it began to fall till the end of August (with one slight rise in the first week of this month). Afterwards the level rose rapidly attaining the maximum height during the first half of September. Then it fell, with slight fluctuations. The fall was pretty rapid up to the first week of November but afterward was slower (though steady).

The fall of the water level is seen to be associated with an increased nitrate content of the soils. The downward movement of the water causes a greater aeration of the soil and ultimately results in a more energetic formation of nitrates.

In the decay and nitrification of the organic materials in the soil, a most important part is taken by the micro-organisms of the soil. These latter are most active within certain ranges of temperature. Hence the temperature of the soil is an important factor in the formation of nitrates in the soil.

Records of the temperature of the soil are not, however, available, but, as Major Leather[1] has shown that there is a close connection between the temperatures of the air and the soil, the 8 a.m. air temperatures are shown in the chart. Till October the temperature has varied between 80° and 85° F. After this there has been a steady fall till the end of the year. After January, the temperature began to rise with occasional fluctuations. It is interesting to note that the fall of the temperature coincides with the beginning of increased production of nitrates which has continued up to the end of the experiment.

It is also interesting to note here another point which shows a close relation between the growth of plants and the nitrification processes going on in the soil. The period during which nitrates began to accumulate in the soils investigated coincides with one of the periods of rapid plant growth in Bihar. In the case of established trees, it has been noted that after a period of vigour coinciding with the early monsoon phase, growth slows down as the monsoon advances. It is not till October that growth begins again[2].

I wish to express my sincere thanks to Mr Howard for the facilities he accorded me in carrying out the above experiments in the Botanical Area at Pusa and for the interest he has taken in the work.

[1] "Soil Temperatures," *Memoirs Dept. Agr. India, Chem. Series*, **4**, no. 2.

[2] A Howard, "Soil Ventilation," *Agr. Research Inst.*, Pusa, Bull. 52, pp. 10, 11.

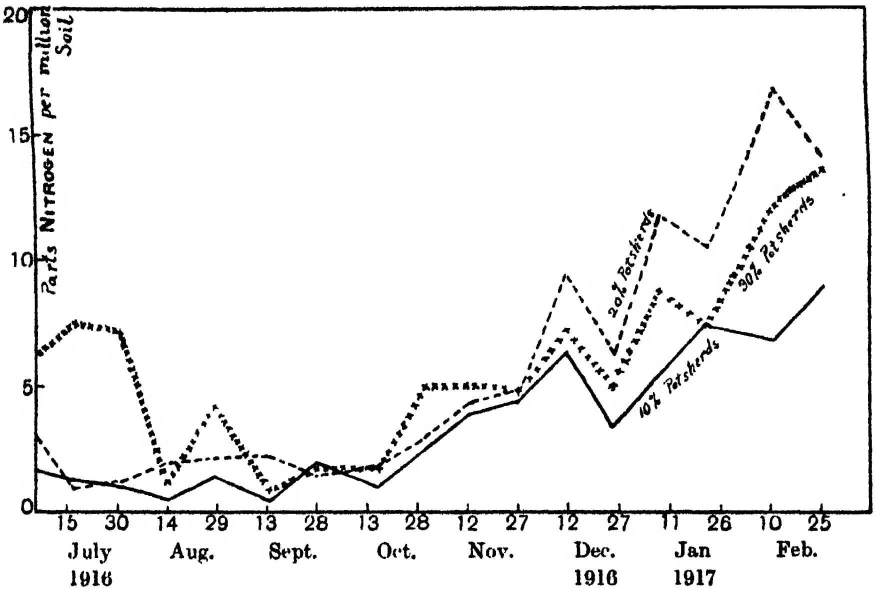

Fig. 1. Nitrates in the soils of 2 ft. gauges

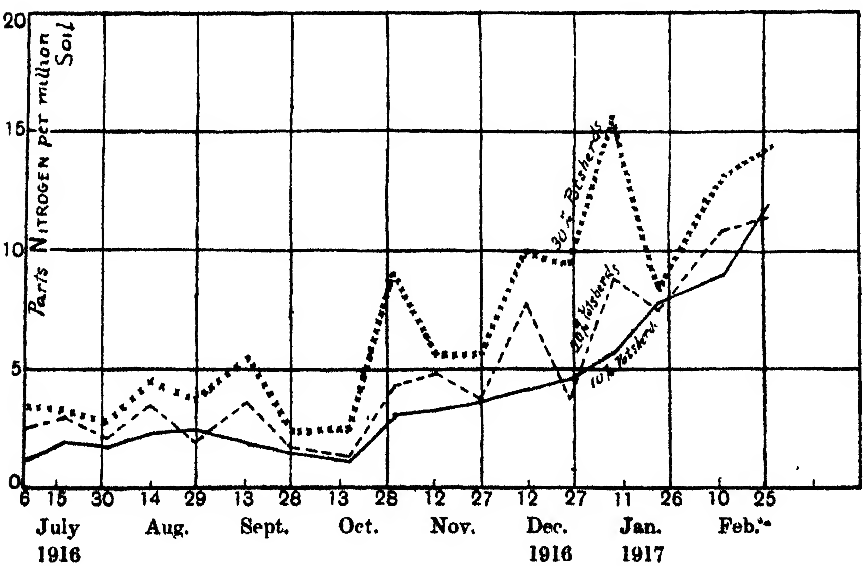

Fig. 2. Nitrates in the soils of 3 ft. gauges.

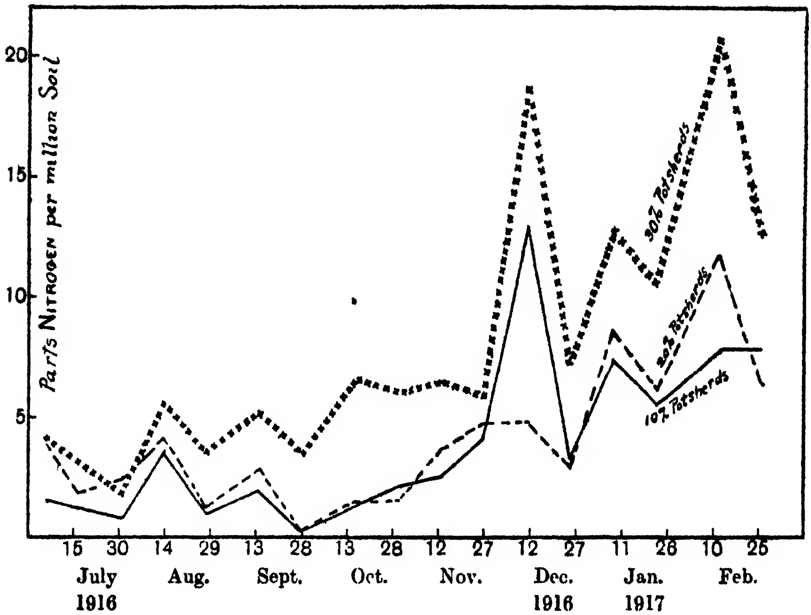

Fig. 3. Nitrates in the soils of 4 ft. gauges.

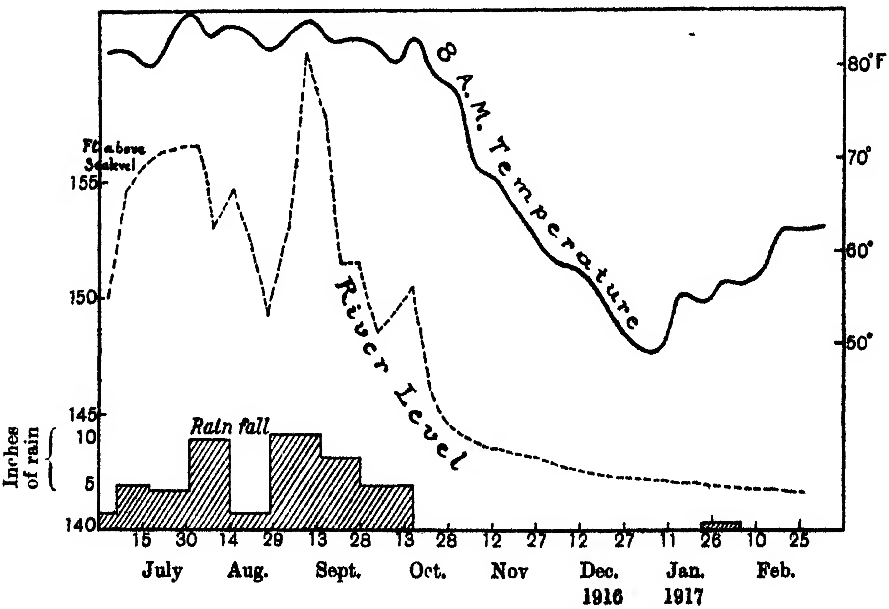

Fig. 4. Air temperature and river level.

THE NON-PERSISTENCE OF BACTERIO-TOXINS IN THE SOIL.

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(With Four Curves in text.)

Since the soil is the abode of a considerable bacterial population and the seat of innumerable bacterial changes it might be supposed that bacterial toxins would occur there. It is of fundamental importance to soil bacteriology to settle this point and to discover whether there is any evidence that such toxins exist, for any depression of cell growth owing to their presence must obviously be reflected in a limitation of plant food production.

If a strict analogy could be drawn between pure culture and soil conditions the question would present little difficulty, but there are several reasons why this is not permissible. Because a pure culture of an organism growing in an artificial medium sooner or later begins to suffer from an accumulation of its own products, it must not be hastily inferred that a similar check occurs in the soil. In the first instance the bacterial flora of the soil is exceedingly complex and many of the observed organisms are largely dependent on the products of the activity of other species or physiological groups. In the second place the soil flora is remarkable for its potency and its power of transforming a considerable range of compounds. Thus there obtains a chain of constructive and destructive processes operating from nitrogen to proteins and back again on the one hand, and from carbon dioxide to cellulose and back on the other, while even such unlikely compounds as phenol, pyridene and carbon bisulphide are not immune from attack. The end products of this collective action are water, carbon dioxide and nitrates which do not accumulate in normal soils but are removed by natural processes. There are of course intermediate products—protein

degradation products, bases, alcohols and organic acids—but it cannot be assumed without definite experimental evidence that they act as toxins to any considerable section of the bacterial population.

During the past seven years several experimenters claim to have obtained evidence of the existence of bacterio-toxins in the soil. Although the possibility of their action in partial sterilisation work was considered by Russell and one of us(1) the first claim to have obtained positive evidence was advanced by Greig Smith(2) who studied the behaviour of a pure culture of *Bacillus prodigiosus* when inoculated into an extract prepared by treating soil with saline solutions. For this purpose the soil was digested with a dilute solution of sodium chloride and the resulting extract after being rendered germ-free by passage through a sterile porcelain filter was divided into two portions, one of which was allowed to remain untreated while the other was heated to 94° or 100° C. for a definite period. The heated extract was then cooled and the two portions were inoculated with a pure culture of the test organism; enumerations of the organisms were made immediately and also after an incubation period—generally 24 or 30 hours.

The results obtained from such experiments showed that when a culture of *B. prodigiosus* was brought into an untreated saline extract of soil, there ensued a marked reduction in the number of cells remaining alive after 24 hours, while a similar culture was capable of appreciable growth when carried into a like extract but which had been previously subjected to heat. Treatment of a soil with chloroform prior to extraction was found to increase the nutritive value of the extract considerably. The extent to which treatment by heat served to improve the suitability of the extract for the life of the organism apparently varied very greatly: in some cases it sufficed to bring the heated extract up to the level of the saline control solution whilst in others the improvement was comparatively small.

The two main objections to which the method is open are, firstly, that the test organism is not a common inhabitant of the soil and the results obtained by its use do not necessarily apply to the typical soil flora; secondly, the assumption is made that all substances in the extract which exert an unfavourable influence on the test organism must necessarily be bacterio-toxins. An equilibrium is said to exist under normal conditions between toxins and nutrients in the soil. Volatile antiseptics are regarded by Greig Smith as effecting a reduction of the water-proofing action of the soil wax, or "agricere" which is assumed to surround the soil particles, and the observed high value, for bacterial

growth, of extracts of soils which have been treated with antiseptics is attributed to an increase of nutrients consequent on such action.

In a later paper the possible significance of toxins both labile and stable, and of antitoxins is discussed.

A further contribution to the subject was made by Bottomley (3) who determined the growth of denitrifying and nitrogen-fixing bacteria when carried into extracts of soil and of manure. It is stated that the former bacteria thrive well whilst the latter do not. We have repeated these experiments with soil but without being able to obtain any corroborative results.

Experiments somewhat similar to those of Greig Smith have been carried out in India by C. M. Hutchinson (4). In connexion with the results of various plate culture experiments he assumes without offering any proof that the varying number of colonies on the different plates is "due to the variation in the content of bacterio-toxin in the several soil extracts from different treatment, so that plates showing large numbers of colonies will result from extracts containing small quantities of toxins and vice versâ." He further assumes that these toxins are destroyed by exposure of the soil to sunlight and air, as well as heat, and also observes that toluene must have a similar effect since the want of toxicity in toluened soil extracts cannot be due merely to redistribution of toxin in the soil. It may be noted however that the latter assumption differs from that of Greig Smith, who does not recognise the destruction of toxins by volatile antiseptics.

C. M. Hutchinson does not advance any experimental data in support of these views, and in fact, in order to account for the somewhat contradictory results it becomes necessary to set up fresh attributes of the various soil extracts. It is stated, for instance, that heating of an extract of *fresh* soil for 15 minutes is sufficient to produce toxins but not to destroy them, while heating for 60 minutes both produces and destroys them. In the case of the extract of *air-dried* soil, it is supposed that the proportion between toxins and organic matter might be such that 15 minutes' heat destroys the toxins initially present and also such as are formed by heating from the organic matter in the soil extract.

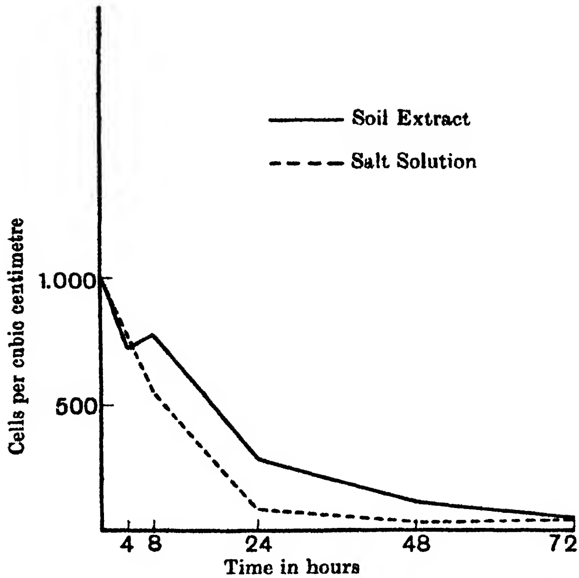
These investigators have not only supposed that their results demonstrated the presence of toxins as normal constituents of the soil, but they argue that the toxins are decomposable by some of the partial sterilisation methods adopted in our investigations, and that therefore the improvement in bacterial growth we observed in partially sterilised soils is due simply to the destruction of toxins. This extension of the

toxin hypothesis involves some difficult and not altogether consistent assumptions as to their properties. It requires the assumption amongst others not only that a number of toxins actually exist in the soil, but that while some are decomposed at temperatures below those which hitherto have been found necessary for toxin destruction, others are sufficiently stable to withstand the relatively high temperatures ordinarily adopted in partial sterilisation.

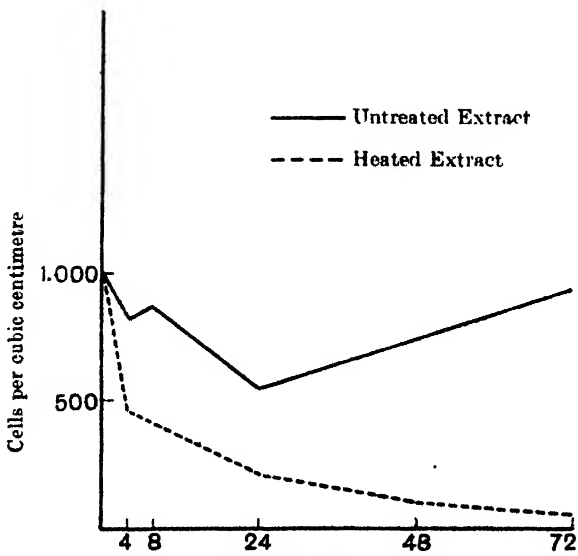
Furthermore the toxins are supposed to be so unstable as to be destroyed by aeration or exposure to light but at the same time they are stable enough to resist decomposition in the soil. Their properties vary in moist and in air-dry soils and while they are sufficiently potent to inhibit growth in untreated soils they are no longer able to do so in those treated with volatile antiseptics, i.e. where the "agricere" is distributed. It becomes necessary to assume that hydrogen sulphide, phenol, etc., destroy them and that the process of salting out in the soil either decomposes them or renders them permanently insoluble. Apart from the various assumptions which the toxin hypothesis necessitates, it is still clear that the extracts of certain soils prepared as suggested by the above investigators produce effects on introduced organisms which are indicative of injurious bodies. Whether or not these bodies belong to the true toxins cannot be decided until more work has been done but the results appeared to be sufficiently striking to warrant a repetition of the work with English soils, not only from its relation to the growth of soil bacteria, but also in regard to the changes induced by partial sterilisation.

I. We have accordingly studied the rate of growth of bacteria in the extracts of a number of different soils and compared it with the rate of growth in physiological salt solution. In some cases a temporary depression occurred, but this was followed by a definite recovery within 72 hours from the time of inoculation. From two of the poorest soils extracts were obtained which showed marked depression of the test organisms and resemble to a certain extent the effects noted by Greig Smith. Comparison with the saline controls shows however that even these extracts are somewhat better and certainly not worse than the controls which were devoid of toxins. These are shown in Curve I.

II. We next attempted to ascertain whether heating the soil extract, which is said by Greig Smith to decompose the "toxin," did as a matter of fact improve the extract for bacterial growth. In all the normal soils tested it did not; on the contrary, it had the opposite effect and led to still lower bacterial numbers (Curve II).

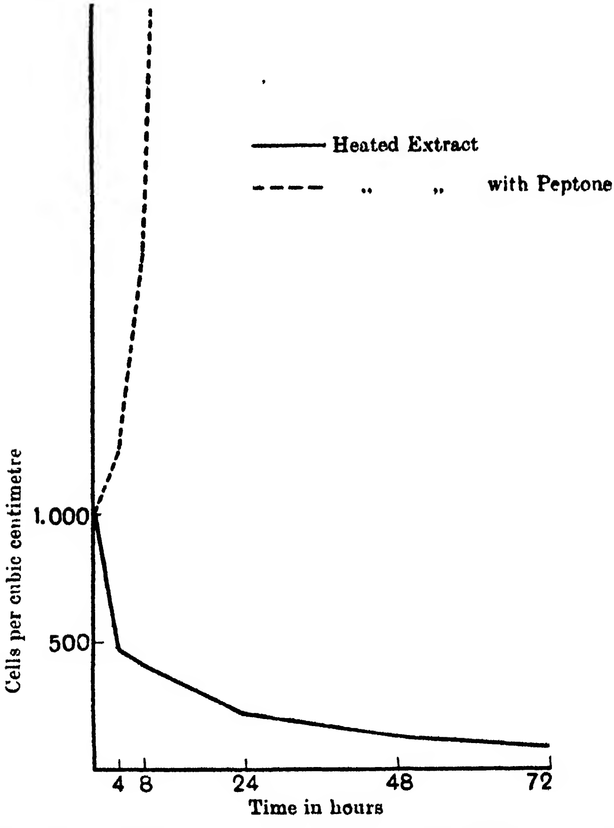


CURVE I. Growth of *Bac. prodigiosus* in extracts of untreated soils (Rothamsted and Millbrook) and in salt solution.



CURVE II. Growth of *Bac. prodigiosus* in untreated and heated soil extracts (mean of 6 soils).

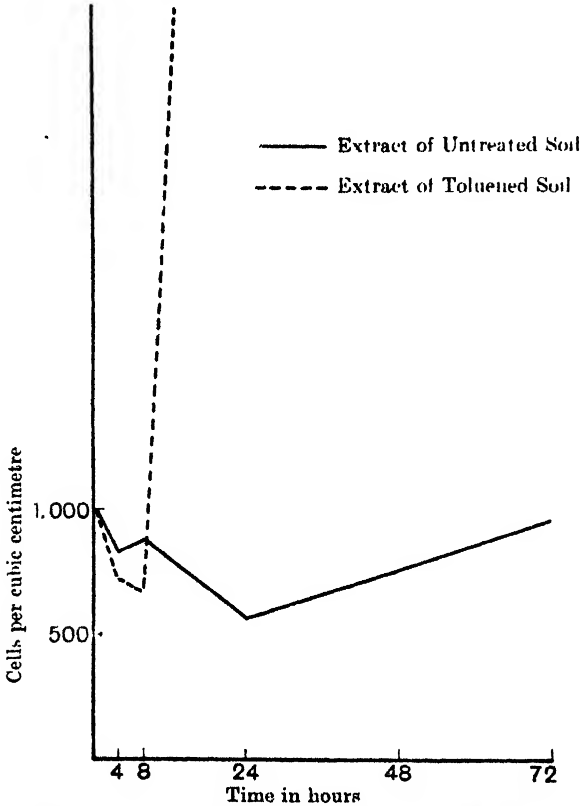
Only in the case of an acid soil—from a barren heath—was any improvement effected, and this we think can be explained more simply on purely chemical grounds. Whereas the untreated extract from this soil after filtration was clear and only slightly straw coloured, it became distinctly turbid and of a reddish brown colour on being subjected to a temperature of 94° for one hour. It thus became apparent that we had



CURVE III. Growth of *Bac. prodigiosus* in heated extract, and heated extract with peptone.

to deal with a soil of the "adsorptively unsaturated" type which has been studied in detail by Daikuhara (5). Such soils on treatment with a neutral salt solution yield an extract containing considerable quantities of iron and alumina, and distinctly acid in reaction. In the case of the heath soil used in our experiments it was possible to demonstrate the presence of such compounds in the extracts. It is difficult, therefore, to avoid the

conclusion that the improvement of the extract on being subjected to heat was more directly connected with the precipitation of these compounds than with the destruction of toxins. Even if one disregards the complications introduced by the peculiar behaviour of this soil on treatment with salt solutions and assumes toxins to have been present in the extracts, it is impossible to contend that this heath soil represents



CURVE IV. Growth of *Bac. prodigiosus* in extracts of untreated and toluened soils.

the normal type. Treatment of the soil with calcium carbonate entirely removes its toxic power.

III. We conclude from our observations that the unsuitability of the heated extracts of normal soils is simply due to the lack of bacterial nutrients. The addition of a minute quantity of food material (equal to six parts of peptone nitrogen per million of the extract) leads to an immediate and complete recovery of its suitability for growth. These results are given in Curve III.

IV. Soils that have been treated with toluene yield extracts capable of carrying far higher numbers of bacteria than the extracts of untreated soils, and this has been assumed to be due to the destruction of toxins. Experiments show, however, that these extracts contain a larger amount of soluble nitrogenous organic matter, to which increased bacterial development can with high probability be ascribed. They differ only slightly if at all from untreated extracts or even saline solutions to which a similar amount of material has been added (Curve IV). The higher nutritive value of extracts of soils which have been treated with volatile antiseptics has been attributed, without proof, however, to an increased extractibility of the soil owing to the localisation of the "agricere"; there is strong presumptive evidence that the soil flora and fauna which succumb to treatment, are contributory sources.

V. The investigation, therefore, gives no support to the view that bacterio-toxins persist in, or are normal constituents of, uncropped soils. Hence the suggestion cannot be accepted that partial sterilisation effects are due wholly or in part to their destruction.

Experimental.

The experiments recorded below were arrived at with the following seven soils, all of which have previously been under investigation either in the laboratory, pot-culture house, or the field.

Allotment Soil. Similar to the garden soil used in partial sterilisation experiments.

Broadbalk Soils. ([*a*] Manured and [*b*] unmanured): under continuous wheat experiments since 1843.

Woburn Soil. (Unmanured): under continuous wheat experiments since 1876.

Millbrook Soil. Light, sandy field soil.

Chelsea Soil. From Chelsea Physic Garden.

Harpenden Common Soil. Heath soil, distinctly acid in reaction.

In the examination of these soils two deviations from Greig Smith's method have been introduced. In the first place we have adopted the use of standard physiological salt solution (0·8 % pure sodium chloride in distilled water), instead of 0·2–0·5 % salt in distilled or tap water; secondly, and in order to permit of the growth of the test organisms being more closely followed, enumerations of bacteria were made directly after inoculation of the extracts as well as after 4, 8, 24, 48, and 72 hours, instead of the single count frequently recorded by Greig Smith. With

each soil four separate determinations were carried out: counts were made in the untreated soil extracts and also in another portion which had been heated to 94° C. for one hour. These were compared with an extract of soil which had been partially sterilised by means of toluene, and lastly we included an extract of untreated soil which had been boiled, and to which minute quantities of food material (peptone) had been added. For the sake of conformity *B. prodigiosus* was used as a test organism with each soil, but with the Broadbalk unmanured and Woburn soils recourse was also had to the use of *B. fluorescens liquefaciens*; this is one of the commonest soil forms, it is non-sporogenic, and owing to the non-formation of zoogloea is suitable for quantitative work.

The data obtained from these experiments are set out in Tables I–VI below, from which it will be seen that the extracts of the various soils differ very appreciably in their suitability for bacterial growth. The results are calculated on a bacterial content of 1000 cells per cubic centimetre at the beginning of each experiment.

TABLE I. *Allotment Soil.*

	Relative Number of Cells in the Extracts after					
	0 hrs.	4 hrs.	8 hrs.	24 hrs.	48 hrs.	72 hrs.
Untreated Extract . .	1000	878	851	797	2730	3594
Heated Extract . . .	1000	887	903	450	340	150
Extract of Toluened Soil .	1000	987	1000	26,870	2,600,000	4,800,000
Heated Extract + Peptone =6 pts. N per million of Extract	1000	1500	3560	167,800	1,300,000	1,200,000

TABLE II. *Chelsea soil.*

	Relative Number of Cells in the Extracts after					
	0 hrs.	4 hrs.	8 hrs.	24 hrs.	48 hrs.	72 hrs.
Untreated Extract . .	1000	1149	1040	770	567	838
Heated Extract . . .	1000	1010	750	472	223	140
Extract of Toluened Soil .	1000	341	329	136,700	18,800,000	34,100,000
Heated Extract + Peptone =6 pts. N per million of Extract	1000	1610	2310	8770	283,000	452,000

TABLE III. *Rothamsted Soil (manured).*

	Relative Number of Cells in the Extracts after					
	0 hrs.	4 hrs.	8 hrs.	24 hrs.	48 hrs.	72 hrs.
Untreated Extract . .	1000	633	?	355	380	660
Heated Extract . . .	1000	290	28	27	39	60
Extract of Toluened Soil .	1000	581	383	232,000	4,100,000	4,000,000
Heated Extract + Peptone =6 pts. N per million of Extract	1000	32	16	16	1080	11,300

TABLE IV. *Woburn Soil (unmanured).*

	Relative Number of Cells in the Extracts after					
	0 hrs.	4 hrs.	8 hrs.	24 hrs.	48 hrs.	72 hrs.
Untreated Extract	1000	—	957	906	660	574
Heated Extract	1000	—	12	31	9	4
Heated Extract + Peptone = 6 pts. N per million of Extract	1000	—	1980	212,000	373,000	373,000

TABLE V. *Millbrook Soil.*

	Relative Number of Cells in the Extracts after					
	0 hrs.	4 hrs.	8 hrs.	24 hrs.	48 hrs.	72 hrs.
Untreated Extract	1000	746	841	222	73	28
Heated Extract	1000	650	716	300	85	33
Extract of Toluened Soil	1000	847	1083	19,170	2,400,000	2,800,000
Heated Extract + Peptone = 6 pts. N per million of Extract	1000	1960	3600	210,000	900,000	

TABLE VI. *Rothamsted Soil (unmanured).*

	Relative Number of Cells in the Extracts after					
	0 hrs.	4 hrs.	8 hrs.	24 hrs.	48 hrs.	72 hrs.
Untreated Extract	1000	697	737	345	142	0
Heated Extract	1000	14	2	2	1	0
Heated Extract + Peptone = 6 pts. N per million of Extract	1000	1180	1380	3810	460,000	579,000

Marked differences appear to exist between the values of the untreated extracts of the various soils and these allow of a useful comparison with the suitability of the original soils for plant growth. From other experiments where some of the soils were used for pot culture the following results were obtained:

Crop grown: Barley	Chelsea	Broadbalk manured	Broadbalk unmanured	Millbrook
Total Dry Matter in grms.	11·54	10·38	5·22	4·68
Total Nitrogen in Crop in grms.	0·090	0·073	0·031	0·024

There is thus some relation between the crop producing power of a soil and the capacity of its extract to support bacterial growth: the more fertile the soil, the more abundant the growth of organisms in the soil extract. Although all the soil extracts showed some reduction in bacterial numbers immediately after inoculation, the organisms appear to have adjusted themselves after 48 hours in the case of the allotment soil and 72 hours in that of the Chelsea and Broadbalk manured soils; their numbers showed, therefore, an upward tendency. With the extracts

of the three other soils there was apparently no such recovery within 72 hours. The latter results are thus in accordance with those given by Greig Smith, and might without further examination be assumed to be due to the presence in the extracts of substances inimical to bacterial growth.

Assuming for the moment that this decrease in numbers is of similar character to those observed by Greig Smith and is due to a like cause to that operating in his extracts, it might be expected that subjecting the extracts to heat would to some extent render them suitable for growth. This, however, is precisely the reverse of what actually takes place with the six soils mentioned above. The value of only one extract is maintained but not increased by heat, while that of the remaining five is so affected that the bacterial numbers after 72 hours are but one-fifteenth of those of the untreated extracts. Hence we are compelled to conclude either (1) that the toxins if present in these six soils must be heat-stable while those in Australian soils are labile; or, (2) that the lack of growth in the untreated extracts of the soils is not determined by the presence of toxins, but by some other factor. Further reference to the latter point will be made later on.

The Extracts of Soils treated with Mild Antiseptics.

The increased fertility of soils which have undergone partial sterilisation by volatile antiseptics has been attributed by Greig Smith to the localisation, on the soil particles, of a soil wax or "agricere." The extraction of nutrients is thus facilitated and the equilibrium which is held to exist between these substances and soil toxins presumably becomes temporarily disturbed. It is difficult to reconcile this view with observed facts; an increased extraction of the soil should also lead to corresponding increases of the soil toxins as well as of nutrient substances, and the equilibrium ought consequently to be maintained.

The results, however, can be explained solely on the basis of a difference of nutritive values without assuming the presence of toxins. Treatment leads to the liberation of soluble organic nitrogen compounds from the soil, and this action is reflected in the high bacterial numbers in the toluened soil extracts. The extracts of three of the soils have been examined and the following results obtained:

	Extractable Organic Nitrogen (mgrm. per kilo of Soil)		
	Allotment	Broadbalk (manured)	Millbrook
Extract of Untreated Soil	1·57	1·15	0·90
Extract of Toluened Soil	5·90	4·20	4·05

Reference to the tables already given (I–VI) will show that in the extracts of three toluened soils the relative numbers of *B. prodigiosus* (i.e. on basis of inoculation with 1000 cells) were found to amount to 4·8, 4·0 and 2·8 millions while the actual contents per cubic centimetre of the extracts were 38, 34, and 20 millions respectively. As these soils in the untreated state normally contain per gram from 8–12 million bacteria (i.e. capable of growth on nitrogenous media) it is evident that the organic substances spontaneously liberated on treatment with antiseptics must in part at least determine the bacterial increases which occur. In fact, some relation appears to exist between the nitrogen thus liberated and the relatively high numbers of bacteria observed in partially sterilised soils[1].

The Effect of Food Substances on Growth in Soil Extracts.

As it appeared possible that the unsuitability of the extracts of untreated soil—and particularly those which had been subjected to heat—might be due largely to the lack of food substances, further determinations were made with extracts of untreated soil which, after being heated

TABLE VII. *Influence of food on Growth.*

Parts per million		Relative Number of Cells in Saline Solutions at 28° after 0 hrs.	4 hrs.	8 hrs.	24 hrs.	48 hrs.	72 hrs.
N.	Saline Control	1000	787	560	76	33	18
1·2	„ + 0·1 cc. peptone solution	1000	766	355	25	23	134
2·4	„ + 0·2 cc „ „	1000	714	460	492	19,050	207 000
3·6	„ + 0·3 cc.	1000	--	422	479	352,000	$1·1 \times 10^6$
4·8	„ + 0·4 cc.	1000	737	500	55,600	$1·65 \times 10^6$	$1·51 \times 10^6$
6	„ + 0·5 cc.	1000	875	1041	$2·2 \times 10^6$	$2·2 \times 10^6$	$2·1 \times 10^6$
12	„ + 1·0 cc.	1000	1070	1990	$4·9 \times 10^6$	–	$5·0 \times 10^6$

for the usual period, received small quantities of peptone solution sufficient to bring the nitrogen content up to about six parts per million of extract. This amount is somewhat higher than that by which the extracts of toluened soils are normally enriched, and was not only sufficient in almost all cases to prevent any decrease in numbers of *B. prodigiosus* in the early stages of incubation, but also resulted in very considerable bacterial growth. In the case of the Rothamsted manured soil a marked decrease occurred, but this was eventually followed by growth after

[1] It is probable that this nitrogen is derived from the organisms (bacteria, algae, protozoa, etc.) which succumb during treatment. *It can be shown, for example, that ciliated protozoa in the trophic state disrupt after* 60–90 *seconds' exposure to the vapour of mild antiseptics.*

48–72 hours. On the basis of these results it is difficult to avoid the conclusion that decreasing bacterial numbers and paucity of the extracts in food materials are intimately connected: this view receives further support from a study of the behaviour of the test organism in saline peptone solutions (Table VII).

From these data several interesting facts emerge. Within the first 24 hours the numbers of living bacteria in the saline control diminished very rapidly, and after 72 hours only about one-fiftieth of the original number continued to exist. Similar behaviour was evident in the most dilute peptone solution for the first 48 hours, after which a slight recovery took place. It is evident, therefore, that this is about the critical dilution which will permit of growth. Higher concentrations of peptone have a pronounced effect in shortening the period during which the organisms tend to diminish, until the solution containing 12 parts of peptone nitrogen per million of saline shows not only no decrease, but a twofold increase within the first 8 hours. Maximum numbers of bacteria were obtained earlier with the stronger solutions than with the weaker, but the final numbers after 72 hours are in most cases roughly proportional to the food supply and the growth curve is therefore practically linear in character. Moreover the results closely resemble those obtained with the various extracts of untreated and toluened soil given above and the following comparison of the growth in the two poorest untreated soil extracts and the saline control shows that the former results might quite well be explained on the basis of food supply.

	Relative Number of Cells after					
	0 hrs	4 hrs	8 hrs.	24 hrs	48 hrs	72 hrs.
Saline Control . . .	1000	787	560	76	33	18
Extracts of Millbrook and Rothamsted Soils (mean)	1000	721	789	283	107	14

In any case the results accord sufficiently well with the prevailing conceptions as to the requisite conditions for cell growth; they differ radically from the high numbers (70,000 and 170,000) which Greig Smith records for some of his saline controls.

The Growth of B. fluorescens liquefaciens *in Soil Extracts.*

As the routine experiments were open to the objection that the test organism *B. prodigiosus* was of comparatively rare occurrence in the soil, further work was undertaken to ascertain the behaviour of a common soil organism under similar conditions, and for this purpose *B. fluorescens liquefaciens* is eminently suitable.

The general method of working was similar to that followed with *B. prodigiosus*, but the experiments were confined to two soils only—the Woburn and Rothamsted unmanured—the extracts of which had given medium and low results respectively with *B. prodigiosus*. The experimental results are given in Tables VIII and IX.

TABLE VIII. *Woburn Soil (unmanured)*: B. fluorescens liquefaciens.

	Relative Number of Cells in the Extracts after					
	0 hrs.	4 hrs.	8 hrs.	24 hrs.	48 hrs.	72 hrs.
Untreated Extract	1000	601	1184	654,000	833,000	1,309,000
Heated Extract	1000	153	147	200,000	—	600,000
Extract of Toluened Soil	1000	558	735	265,000	4,100,000	4,800,000
Heated Extract + Peptone	1000	3760	8080	15,600,000	16,400,000	24,000,000

TABLE IX. *Rothamsted Soil (unmanured)*: B. fluorescens liquefaciens.

	Relative Number of Cells in the Extracts after					
	0 hrs.	4 hrs.	8 hrs.	24 hrs.	48 hrs.	72 hrs.
Untreated Extract	1000	721	616	72	452,000	1,163,000
Heated Extract	1000	31	3	1	1870	514,000
Extract of Toluened Soil	1000	723	605	243,000	1,100,000	?
Heated Extract + Peptone	1000	970	1840	854,000	1 780,000	1,840,000

These results are significant in that they show the vital importance attaching to the choice of organisms for the test; with an extraneous organism such as *B. prodigiosus* effects are induced which might without further inquiry be interpreted as being due to the action of toxins: with a soil organism such as *B. fluorescens liquefaciens* no such interpretation is possible. Whilst the relative numbers of the former organism in the Woburn soil extract decreased within 72 hours to 574, those of *B. fluorescens liquefaciens* steadily increased to over 1·3 millions. Still more striking results were obtained with the Rothamsted soil and having regard to these facts it is difficult to advance any satisfactory explanation on the basis of the occurrence of toxins. The decrease in the value of the extracts on heating, although considerable, is not so pronounced as in the case of *B. prodigiosus*; this conforms with the known difference in proteolytic power of the two species.

The experimental evidence obtained from the study of the above six soils may therefore be summarised as follows:

(*a*) Extracts of untreated soils and especially of poor soils are relatively unsuitable for the growth of *B. prodigiosus*.

(*b*) The low value of such extracts is still further decreased when the extracts are subjected to heat.

(*c*) Toluened soils yield extracts containing increased amounts of soluble organic nitrogen and are consequently of greater value for growth than the untreated soil extracts.

(*d*) The low value of heated extracts may be fully restored by the addition of suitable quantities of peptone.

(*e*) The type of result obtained varies largely with the character of the soil itself and according to the test organism, but in no instance has it been possible to obtain definite indications of the presence of toxins either with fresh samples of soil from the field or in those stored in the laboratory in a moist condition.

The Extracts of "Abnormal" Soils.

In addition to the six soils mentioned in the foregoing pages, a seventh sample was examined in the prescribed manner and as the results are of such a manifestly different order it appeared desirable to give them separate consideration.

Whilst the majority of the soils studied are normally under cultivation, the remaining soil was derived from heath land and is generally under a cover of either gorse (*Ulex europaeus*) or of sorrel (*Rumex acetosella*). As might be presumed from the occurrence of this type of vegetation, the soil is markedly acid in character and is unsuited for the growth of cereals, e.g. barley. In spite of this, the extract of the untreated soil was found to be more suitable for the growth of *B. prodigiosus* than those of the relatively more productive Millbrook and Rothamsted (unmanured) soils. Considerable growth occurred in the heated extract (the relative numbers were 450,000 after 72 hours) and thus approached more closely to the behaviour observed by Greig Smith.

TABLE X.

	Relative Number of Cells of *B. prodigiosus* in the Extracts after					
	0 hrs.	4 hrs.	8 hrs.	24 hrs.	48 hrs.	72 hrs.
Untreated	1000	1033	1000	734	303	204
Heated	1000	1365	2133	52,000	307,000	450,000
Toluened	1000	676	639	120	24	2
Heated Extract + Peptone .	1000	3180	8020	1.854,000	3,330,000	3,540,000

The addition of peptone to the heated extract is followed by the usual response, but the lack of growth in the extract of toluened soil is peculiar and at present inexplicable. In contradistinction to the extracts of the other soils examined, the extract of this soil became, on being

subjected to heat, distinctly turbid owing to the formation of a finely divided yellowish brown precipitate, which was found on examination to contain appreciable quantities of iron. The occurrence of such compounds in saline extracts of acid soils has been frequently noted and has been the subject of investigation by Daikuhara. The amount of interaction which takes place between the soil and neutral salt solutions appears to be determined by the amount of adsorptively unsaturated substances, by the concentration of the solution, by the salt used, and results in the passage of appreciable quantities of iron and aluminia compounds into acid solution. The effect of these compounds on introduced organisms has not yet been ascertained, but it is noteworthy that the only soil found by us to yield acid extracts, also exhibited effects corresponding to those which were interpreted by Greig Smith as being due to toxins. How far these two facts are directly connected must for the present remain an open question, but it might here be mentioned that Greig Smith records the generally acid character of Australian soils but without attempting to correlate soil reaction and toxicity of the extracts. It is possibly in some such direction that an explanation of the observed phenomena may be sought, but in any case the use of saline solutions for the preparation of the extracts by introducing the interchange of bases appears to complicate the question at issue.

As this soil was the only one of the seven examined which gave extracts indicative of the presence of "toxins," it appeared of interest to determine what relation, if any, existed between acidity and "toxicity." With this in view two equal samples of 80 grams of soil were digested (*a*) as control, in 1600 c.c. of water saturated with carbon dioxide, and (*b*) in 1600 c.c. of a solution of calcium carbonate likewise in water saturated with carbon dioxide. After 4 hours' digestion the soils were dried on a Buchner filter and in case the bacterial flora should have been adversely affected by exposure to the carbon dioxide, the two portions of soil were inoculated by means of an aqueous extract of normal soil. The soils were then incubated and examined after 24 and 48 hours. The results of enumerations were as follows:

	Bacteria per gram of soil after	
	24 hrs.	48 hrs.
Untreated Soil . . .	4,100,000	2,700,000
Soil neutralised with $CaCO_3$	41,800,000	83,500,000

The rapid increase in the numbers of bacteria in the soil saturated with calcium carbonate cannot be attributed to the extraction of toxins

from the soil during digestion, for the control soil was digested with an equal amount of water. The absence of any apparent restriction of bacterial growth in the former soil, even within the first 24 hours after treatment, makes it extremely improbable that toxins were at all operative in this soil. As might be anticipated, the improvement in the soil conditions is not confined to bacterial growth alone, but is also reflected in a greatly increased crop dry matter production. In this respect the following data obtained from pot experiments may be of interest.

	Harpenden Common Soil: Crop, Barley	
	Untreated Soil	Soil + $CaCO_3$
Dry matter in Crop	1·05 grams	17·65 grams

The limitation of bacterial and plant growth appears therefore to be determined more by chemical than bacterial conditions.

In submitting this explanation of the observed facts we do not, however, wish to imply that the toxicity of the extracts is solely due to the acidity of the soil itself. It is in fact conceivable that in soil possessing an acid reaction, the protein degradation changes may be arrested and incomplete, just as the process of nitrification in these soils is restricted, or the decomposition of carbonaceous residues only partially takes place.

The Behaviour of B. prodigiosus *towards its own Products of Growth.*

In conjunction with the foregoing experiments, which are based on the supposed susceptibility of *B. prodigiosus* to the by-products of the mixed bacterial flora of the soil, we also determined the behaviour of this organism towards its own by-products.

As the conventional media, such as dextrose broth, are unduly concentrated and are apt to yield complex results on account of acid formation from the sugar, recourse was had to the use of an extract of toluened Chelsea soil, which had been found to support vigorous growth of the organisms. The extract was prepared by the usual method and inoculated with a suspension of *B. prodigiosus* in saline solution. Growth was allowed to take place at 28° C. for 10 days, by which time no further increase could be found. The extract or culture was then divided into two portions, one of which was filtered by passage through a Berkfeld candle, while the other was pasteurised at 70° for half an hour. The two portions were then inoculated with 4200 and

2300 cells per c.c. respectively and again incubated. The results of the analyses subsequently made are subjoined:

Secondary Culture	At Beginning	1 day	2 days	4 days	7 days	8 days	10 days
A. Pasteurised Extract	1000	37,500	—	—	—	—	—
B. Filtered Extract	1000	381	571	2140	7850	11,670	3330

The superiority of the pasteurised over the filtered extract might, at the first glance, be assumed to be due to the destruction of some labile toxic substance in the former extract, but further investigation requires a modification of this view. On the incidence of decreasing numbers in portion B after 10 days, this extract (or culture) was again filtered and divided into three fresh portions, one of which remained untreated, a second was pasteurised at 70° for 30 minutes, and the third received the usual addition of peptone. Counts which were made on commencing the experiment, and also after 38 hours, gave the following results:

Tertiary Culture	At Beginning	After 38 hrs.
Untreated	1000	less than 1 cell per c.c.
Pasteurised . . .	1000	2
Untreated + Peptone .	1000	65,000,000

In this instance pasteurisation failed to improve the value of the filtered extract, and we must therefore conclude that the difference which was found to exist between A and B above, was not determined by the destruction of a toxic body by pasteurisation but by the food substances of the preceding generation of cells which were retained in the liquid. Repeated growth and removal of the cells of two generations apparently suffices to reduce the extract of toluened soil to a condition unsuitable for further growth. The fresh extract or primary culture (Table II) showed a relative increase of 136,700 after 24 hours: the secondary culture gave only 381 cells, whilst the tertiary culture showed practical extinction of the organisms after upwards of 40 hours.

The addition of minute quantities of peptone to these supposedly toxic extracts sufficed to bring the relative content to upwards of 65 million, and thus fully confirmed the results obtained from the study of the extracts of untreated soils.

Some indication of the presence of inhibiting substances is however afforded by experiments in which portions of the extract used for the tertiary culture were diluted to varying degrees.

	0 hrs.	24 hrs.	48 hrs.
Extract : Water, 2 : 1	1000	9	less than 1 per c.c.
" : " 1 : 1	1000	400	920
" : " 1 : 2	1000	—	11,300*
Undiluted Extract (boiled for 2 hours) .	1000	88	8

* After 36 hours.

Dilution leads to a marked improvement in the suitability of the filtered culture for growth, and is thus distinctly more effective than treatment by heat. Hence it bears little relation to the alleged soil toxins or to the inhibitory substances formed by *B. fluorescens liquefaciens* and *B. coli* which were found by Rahn (6) to be incapable of passing through a porcelain filter and were readily destroyed by heat.

SUMMARY.

The experimental results submitted in the preceding pages may be summarised as follows:

Seven English soils have been examined in order to ascertain the validity of Greig Smith's claim that partial sterilisation effects may be due to the destruction of bacterio-toxins in the soil. The results obtained with six of these soils show that:

1. The untreated extracts of these soils varied largely in their suitability for the growth of the test organism (*B. prodigiosus*). In some instances vigorous growth occurred; in others the numbers of introduced organisms fell to a minimum.

2. Treatment of the extracts by heat (which was supposed to result in the destruction of "toxins") invariably led to still further bacterial decreases.

3. Extracts of soils treated with antiseptics (which are not supposed to destroy "toxins") were on the whole more favourable for growth than those of untreated soils. Such extracts were found to have appreciably more organic nitrogen compounds than extracts of untreated soils.

4. The addition of minute quantities of peptone to unsuitable extracts sufficed to convert them into favourable media.

5. Extracts of the two poorest untreated soils were tested with a common soil organism (*B. fluorescens liquefaciens*). No evidence of toxicity could be obtained; on the contrary, very abundant growth occurred. Results obtained by the use of an extraneous organism, such as *B. prodigiosus*, must be accepted with reserve.

6. The curve of diminished numbers of bacteria in poor untreated soil extracts is practically identical with that obtained when bacteria are introduced into pure salt solutions: the decreases are symptomatic of starvation.

7. The only soil which gave extracts similar in behaviour to those reported by Greig Smith was an acid heath soil. The value of the extract of this soil was distinctly increased after the extract had been subjected

to heat. Concomitantly, acid iron and alumina compounds which are removed from the soil by the action of the saline solution are thrown out of action. The "toxicity" of this soil can be rapidly (within 24 hours) and effectively removed by treatment with calcium carbonate.

8. Alternate inoculation and removal of the bacterial growth by filtration rapidly yields an extract unfavourable for the growth of *B. prodigiosus*: this is in part due to the impoverishment of the extract in food material, and also to the formation of some substance inimical to growth. This body is capable of passage through a porcelain filter and is heat stable: it therefore appears to have little in common with the inhibitory bodies described by Rahn as occurring in cultures of organisms such as *B. fluorescens liquefaciens* or *B. coli*, nor does it resemble, in its relations to heat, the toxins which are alleged to occur in the soil.

9. Although it is possible, under well-defined conditions, to induce the formation of bacterio-toxins in culture solutions, there is no evidence to show that these are likely to possess importance in the phenomena of partial sterilisation of soil.

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(*Received January 17th*, 1918.)

PHEASANTS AND AGRICULTURE.

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Natural Sciences Tripos and Diploma in Agriculture of the University of Cambridge.

WITH AN INTRODUCTION BY

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Food of Pheasants.

The following Report on the contents of 311 pheasant crops presents the results of a very laborious investigation undertaken by Miss A. F. C.-H. Evershed during 1915 under my direction. Only those who have themselves attempted it will fully appreciate the amount of time and trouble involved in identifying the fragments of insects and plants met with in such a research.

It is important that two things should be borne in mind. In the first place the Report purports to be a statement of facts; deductions are left for the individual reader to draw. In any case generalisations are difficult, since it is impossible satisfactorily to balance, say, wireworms against stolen wheat grains, but beyond this the circumstances of the particular bird have to be taken into account. For example, the birds numbered XII 4 and XXIX 6 both contained a large number of oat grains, but they were far from equally guilty. The former, shot on March 30, had raided the newly-sown crop, while the latter, shot on August 29, had doubtless obtained the grain from stubble.

In the second place, since the Report is concerned simply with crop contents, and does not include any observations in the field, it can obviously have no bearing on cases where it is alleged that pheasants have done harm by wantonly pulling up grass or seedlings which they have not eaten.

Each reader, then, must be left to draw his own conclusions from the statement of facts presented below, but it may perhaps be helpful if we briefly analyse the nature of the food of the pheasant month by month as far as it is revealed by the crops sent for examination.

January, 135 crops, the contents of 40 being sent in one jar and of 50 in another. Beyond hand-fed grain, the bulk of the food consisted of

the seeds of weeds. Early in the month, pine needles and the tubers of the lesser celandine occurred plentifully, and later, spangle galls from oak-leaves were generally found. The 40 crops contained between them 64 germinating wheat grains, probably taken by only three or four of the birds, for of the separate crops only three included any, and the whole 135 crops did not average a grain apiece.

February, 8 crops, of which only three contained much food of any sort, chiefly weeds (lesser celandine, dead nettle, etc.) and a little grass and clover. One bird, VIII 8, had done good service by eating 100 plantain seeds and 2956 grubs of a Bibio fly. These grubs, to some extent injurious as root feeders, are gregarious in habit, and the pheasant had apparently had the luck to come across a nest or two of them.

March, 26 crops. Not a very good month for the reputation of the pheasant, which begins to shew too great a partiality for grass and clover, and also steals grain. Only one crop, IX 1, however, is noted as containing "a good deal" of clover, and this has some compensation in 1806 seeds of spurrey. One has 30 grains of germinating wheat, and one 75 grains of germinating oats, while 370 grains of germinating oats are found in the crop of a bird shot in a "grass and barley" field. Bibio grubs occur again.

April, 22 crops, 20 of them innocent or beneficial. Two of the birds are, however, distinctly blameworthy, XVII 2, containing much clover, 126 grains of germinating barley and 97 sainfoin seedlings, against which it has only two wireworms to shew; while XVII 3 has much sainfoin and 36 germinating barley grains.

The insect food becomes more plentiful, and click beetles (the parents of the wireworm) and plant-lice figure in the diet, in which also a great number of chickweed capsules are noticeable.

May, 16 crops. The food is very miscellaneous—a little grass and clover mixed with the leaves of weeds; many weed seeds, especially chickweed; a good many pine seeds and various insects, including garden chafers. One of the birds, XX 3, had, however, raided a sainfoin crop, and the crop of another, XXI 6, contained a reprehensible amount of clover.

June, 7 crops. Too small a number to warrant much generalisation. Their contents were chiefly bulbous buttercup stems and seeds, together with insects.

July, 16 crops. The food is almost entirely weed seeds, insects and cereal grains. Towards the end of the month, a good deal of the grain is clearly not hand-fed, and, in estimating the relation of the

pheasant to agriculture, we have to take into account the probable source whence the grain has been obtained. The fact that a good deal of grain is found in crops from July 21 onwards, when the corn is just ripening for harvest and during and after harvest, suggests that its source is lodged patches of corn and stubble.

August, 22 crops. Besides a good deal of grain, probably from stubble, there is a miscellaneous vegetable diet of blackberries and weed seeds of various species. One bird, XXVI 5, had taken "much" clover, and 3000 seeds of rye grass. Among the insects eaten, ants are conspicuous.

September, 16 crops. The vegetable diet is much the same as for August, except that more of the crops contain a small amount of clover. A good many crane-flies (parents of the "leather-jacket") figure among the insects eaten.

October, 18 crops. The favourite food seems to be the seeds of chickweed and black bindweed, and, towards the end of the month, acorns and spangle galls from oak-leaves. Two birds (XXXII 3 and XXXII 4) had, however, stolen considerable quantities of germinating rye—144 grains and 178 grains respectively.

November, 8 crops, mostly containing weed seeds (especially *Persicaria*, *Carex* and bindweed, but also many others) and spangle galls from oak-leaves. None of the birds had done any harm.

December, 16 crops. The food is the same as for November with the addition of a considerable number of acorns.

CECIL WARBURTON.

SCHOOL OF AGRICULTURE,
CAMBRIDGE.
March 20th, 1916.

PHEASANTS AND AGRICULTURE.

This investigation as to the food of pheasants was carried out at the request of the East Anglian Game Protection Society, whose members supplied the pheasants' crops for examination throughout the year 1914. My thanks are due to Mr Sidney Armstrong for help in the identification of weed seeds and to Mr Cecil Warburton for checking my determination of the insects.

During this work the crops of 311 pheasants were examined. Unfortunately on two occasions the contents of many crops (40 in one case, 50 in another) were mingled, but this was thought to be unsatisfactory and thereafter the crops were kept separate.

Birds were not sent, but only their crops with their contents, and the method of dealing with these was in the main that described by Hammond in his paper entitled "Investigation concerning the Food of certain Birds," published in the *Journal of Agricultural Science*, Vol. IV, Pt 4, p. 382. The senders were supplied with forms on which to record certain facts such as sex, when and where killed, etc. Jars of spirit were also provided into which the duly labelled crops were immediately put, and which were sent to Cambridge when full. They are indicated in Tables II and III by the Roman numerals. Besides cereal grains and agricultural seeds six kinds of food were recognised—roots and stems of weeds, weed leaves, flowers, weed seeds, insects and "miscellaneous." The "miscellaneous" category embraced such odds and ends as stones, shot, and an occasional worm, slug or spider.

The roots and stems were often unidentifiable with certainty, but the following were recognised: bulbs of lesser celandine, stems or roots of dock, horsetail, and bulbous buttercup.

The leaves included grass, pine needles, lesser celandine, thistle, ground ivy, cabbage, clover, medick, ribwort, plantain, species of Polygonum, sainfoin, daisy, dead nettle, scarlet pimpernel, chickweed, bedstraw, buttercup, goose-grass, species of Potentilla, geranium, bracken, beech, sheep's sorrel, campion, poppy, blackberry, convolvulus, hawthorn, together with many fragments which were beyond identification.

Among the flowers were noted buttercup, hazel catkins, sedges, gorse, woodruff, dandelion, sainfoin, campion, knapweed, poppy and numerous fragments of inflorescences of composite weeds not easily distinguishable.

Among the weed seeds (and fruits) were noted privet berries and seeds, larch, slender false brome, fat hen, hawthorn, knot-grass, meadow grass, goose grass, field pansy, ribwort, wild hemp, nettle, campion, Persicaria, Rubus sp., black bindweed, sheep's sorrel, acorns, ash, pine, hedge parsley, spurrey, sedges, rush sp., chickweed, meadow grass, vetch, sandwort, mouse-ear, shepherd's purse, buttercup, scarlet pimpernel, sheep's fescue, hard fescue, meadow fescue, charlock, enchanter's nightshade, field madder, greater plantain, silver birch and a few others.

The insects were very various. I include here the spangle galls of the oak, of which pheasants seem to be particularly fond, apparently relishing the enclosed grub. Other insects were earwigs, thrips, springtails, Psocids, Carabid beetles and grubs, Staphylinid beetles and grubs, many weevils, notably Sitones, Telephorid grubs, click beetles and wireworms, chafers, a few ladybirds, flea-beetles, saw-flies, ants, surface

caterpillars, swift-moth caterpillars, grass-moth caterpillars, the larvae and puparia of various muscid flies, very many Bibio grubs, a few imagos of Empis, Tipula, Thereva and Asilus, Capsid bugs, green-fly, frog-hoppers and Scutellarid bugs.

The following analysis may be of interest. Seeing that there were 311 crops in all and that 90 of them (sent in lots of 40 and 50 with the contents mingled) could only be treated as two, there are 223 separate consignments. Table I shews the number which contained each of the above six categories of food, arranged in the order of their occurrence:

TABLE I.

Leaves	Weed seeds	Insects	Misc.	Roots and stems	Flowers
150	134	104	97	50	39

That is, 150 crops contained the leaves of plants, many of them containing other kinds of food as well.

119 crops contained cereal grains or agricultural seeds of some sort, but a careful consideration of them, as I shall explain later on, seems to reduce the real cases of injury to 12 birds, there being good evidence in the remaining cases that the corn was either hand-fed or picked up in the stubble, and the other farm seeds obtained from various unimportant sources and not from the growing crop. If I am right in this, and if the offence of stealing from the stubble is admitted to be a trivial one, the damage done by pheasants to growing agricultural crops seems almost negligible.

Unfortunately the number of crops received month by month was very unequal, so that while we have the evidence of 135 crops as to what the birds were feeding on in January, our knowledge of their June operations rests on the evidence of seven crops only. Still no month is unrepresented, and a full record has been kept of the crop contents in each case. They are given in Table II.

Table II will indicate the method adopted in recording the crop contents of each bird, and the general nature of the food during each month of the year, but it is desirable to examine closely all the cases of apparent injury to farm crops. In Table III are to be found all the birds whose crops contained agricultural seeds other than hand-fed grain, with comments on each case. The majority of them prove to be of no importance, but attention is called, by the use of heavy type, to instances of genuine injury.

TABLE II. *Shewing the contents of the crops for each month of the year.*

JANUARY

No.	Sex	District	Locality	Roots and stems	Weed leaves	Flowers	Agricultural seeds	Weed seeds	Insects	Misc.
I 1	Cock	Croxton, Cambs.	—	—	pine needles 24 traces of grass and thistle	—	wheat 784‡ maize 10‡ barley 12‡ oats 2‡	—	—	moss
I 2	Cock	,,	—	—	pine needles 20 grass*	—	wheat 614‡ barley 8‡ oats 4‡ maize 2‡	privet 4	—	moss 1 shot
I 3	Hen	,,	—	—	grass* unidentified 3	—	wheat 208‡ maize 4‡	—	—	—
I 4	Hen	,,	—	—	grass and knot-grass*	—	barley 231‡ oats 84‡ black oats 8‡ wheat 15‡ maize 12‡	—	—	—
I 5	Hen	,,	—	—	pine needles 15 grass*	—	wheat 263‡ barley 27‡ oats 8‡ maize 2‡	—	—	1 shot
I 6	Cock	,,	—	—	pine needles 8 grass*	—	wheat 783‡ barley 47‡ oats 27‡ black oats 23‡	slender false brome 3	—	—
I 7	Cock	,,	—	lesser celandine tubers 456	ivy 4 ground ivy 6 lesser celandine 5 other 6	—	—	larch 24	—	moss*
I 8	Cock	,,	—	—	—	—	—	privet berries 7 privet seeds 124	spangle galls 8	1 stone
I 9	Hen	,,	—	—	grass* unidentified 5	—	barley 63‡ oats 22‡ black oats 24‡ wheat 8‡ maize 2‡	slender false brome 5	—	—

* A trace. ‡ Hand-fed.

JANUARY

I 10	Hen	,,	—	—	—	—	wheat 23‡ maize 6‡ germinating barley 1	privet 2	—	—
I 11	Hen	,,	—	—	pine needles 12 grass*	—	wheat 500‡ oats 7‡ black oats 2‡ barley 12‡	slender false brome 5	—	—
I 12	Hen	,,	—	—	pine needles 10 grass*	—	wheat 337‡ oats 6‡ black oats 10‡ barley 31‡	slender false brome 1	—	1 shot
I 13	Hen	,,	—	—	pine needles 4 grass*	—	wheat 292‡ oats 11‡ black oats 3‡ barley 28‡	slender false brome 1	—	moss*
II 1	Hen	Norfolk	white turnips	—	grass*	—	—	—	—	—
II 2	Cock (old)	,,	ley ¼ mile from feed	—	clover†	—	wheat 1	—	—	—
II 3	Cock	,,	swedes near marsh	horsetail stem 57	buttercup 24 grass* other fragments	—	—	—	—	cone of horsetail
II 4	Cock	,,	barley stubble	? root fragments	grass 8 thistle 20	—	—	fat hen 476 hawthorn 2 knot grass 10 grass§ speedwell 2 bindweed 1	Staphylinid beetle pupae 3	soil
II 5	Cock	,,	young wheat	—	grass*	—	—	—	—	—
II 6	Cock (old)	,,	park land 50 yards from feed	root fragments ? dandelion	cabbage clover medick ? thistle	—	—	goose grass 2 field pansy 8 meadow grass	puparium of fly	—
II 7	Cock	,,	marsh	lesser celandine 17	grass*	—	maize 265‡ wheat 278‡	—	—	moss* stones 3

* A trace. † A little. ‡ Hand-fed. § Much.

TABLE II (continued)

No	Sex	District	Locality	Roots and stems	Weed leaves	Flowers	Agricultural seeds	Weed seeds	Insects	Misc.
II 8	Cock	Norfolk	wood near feed	—	grass*	—	maize 30‡ wheat 5‡	—	—	—
III A (40 crops)	—	Roxham and Fordham, Norfolk	fen	? dandelion old and young tubers of lesser celandine	grass buttercup clover† ribwort, plantain Polygonum sp. lesser celandine	—	maize 50‡ wheat 143‡ barley 274‡ mustard 12‡ germinating wheat 64	plantain 694 hemp nettle 360 false brome 133 goose grass 60 Persicaria 55 Silene 35 knot grass 95 bindweed 52 Rubus spp 97 fat hen 22 other 4	Bibio larvae 101 spangle galls 4 surface caterpillar 1 Carabid beetle 1 Staphylinid grub 1	shot soil stones horsetail cone 1 wood-louse
III B (50 crops)	—	Ryston and Bexwell, Norfolk	highland	lesser celandine 38 bulbous buttercup 9 horsetail 9 many unidentified	grass, clover, and fragments of dead nettle and fern	—	maize 812‡ barley 3‡	sheep's sorrel about 514,500 ash 231 Rubus spp. 176 plantain 52 acorns 36 fat hen 4 bindweed 8	spangle galls 155 weevils 7 larvae of swift-moth and grassmoth and of blow-fly	sand stones soil 1 snail
IV 1	Cock	Chelmondiston, Suffolk	woods where food is laid	—	grass*	—	wheat 703‡	—	—	moss
IV 2	Cock	„	„	—	grass*	—	wheat 391‡	—	—	—
IV 3	Cock	„	„	fragments unidentified	fragments	—	wheat 36	—	—	some vegetable matter not identified
V 1	Cock	Hengrave, Hampton, and Risby, Suffolk	near wood where food is laid	lesser celandine 2	clover* other fragments	—	maize 119‡	Rubus sp. 16 pine 14	—	stones 2
V 2	Cock	„	„	—	grass*	—	maize 105‡	—	spangle galls 2	—
V 3	Cock	„	„	—	unidentified fragments	—	maize 47‡	Capsules of rush sp. 36 Rubus sp. 16	spangle galls 4 Dipt. larvae 2	1 snail

* A trace. † A little. ‡ Hand-fed.

JANUARY	V 4	Cock	,,	,,	—	grass*	—	maize 69‡	—	—	—
	V 5	Cock	,,	,,	root fragments unidentified	clover medick with some grass and sainfoin	—	—	hedge parsley 600	spangle gall 1	1 shot
	V 6	Cock	,,	,,	1 small rhizome	—	—	maize 140‡	3 seeds?	—	—
	V 7	Cock	,,	,,	—	grass*	—	maize 224‡	—	—	—
	V 8	Cock	,,	,,	—	—	—	maize 112‡	—	spangle galls 4 Capsid bug 1	moss
	V 9	Cock	,,	,,	—	grass*	—	maize 224‡	—	—	—
	V 10	Cock	,,	,,	—	—	—	maize 61‡	—	—	stones 2
	VI 1	Hen	Cambs.	arable	rhizome fragments 5	—	—	wheat: germinating 11 hand-fed or picked up 35	—	Staphylinid beetle pupa 1	soil
	VI 2 and 3	Hens	,,	,,	lesser celandine 1 fragments 3	grass* fragments of lesser celandine and buttercup	—	wheat: germinating 33 hand-fed or picked up 302	acorns 2 black bindweed 1	Telephorid larvae 2	pulpy vegetable matter
	VII 8 crops	5 cocks 3 hens	Thelveton, Norfolk	arable, near wood where feed was laid	many fragments unidentifiable	clover 39 other 3	—	maize 278‡ wheat 393‡ barley 144‡ peas 12	slender false brome 16 ash ½	—	shot 1 stones 3
FEBRUARY	VIII 1	Cock	Bexwell, Norfolk	highland	—	celandine 34 buttercup† grass† clover† daisy† sheep's sorrel† dock†	—	—	acorn 1	spangle galls 18	moss
	VIII 2	Cock	,,		root fragments 20	—	—	—	acorns 2	spangle galls 2	—

* A trace. † A little. ‡ Hand-fed

TABLE II (*continued*)

Month	No.	Sex	District	Locality	Roots and stems	Weed leaves	Flowers	Agricultural seeds	Weed seeds	Insects	Misc.
February	VIII 3	Cock	Bexwell, Norfolk	highland	—	—	—	maize 118	—	—	—
	VIII 4	Cock	,,	,,	—	—	—	germinating beans 6	—	—	—
	VIII 5	Cock	,,	,,	root fragments 8	celandine 13 dead nettle 8 buttercup grass† clover†	buttercup 1	maize 2	—	—	—
	VIII 6	Cock	Roxwell and Fordham, Norfolk	fen	—	—	—	germinating beans 25	—	Bibio larvae 3	—
	VIII 7	Cock	,,	,,	—	—	—	—	hemp nettle 3	Bibio larvae 12	—
	VIII 8	Cock	,,	,,	—	dock grass† clover†	—	—	ribwort plantain 100	Bibio larvae 2956	—
March	IX 1	Cock	Suffolk	ley near wood	dock and other roots	clover§ grass dock plantain Polygonum	—	wheat: hand-fed 3 germinating 1	spurrey 1806 other seeds 28	—	stones 2 soil
	IX 2	Cock	,,	wheat	lesser celandine 22	grass clover fern celandine and others	hazel catkins 2	—	goose grass 53 ash 2	—	—
	IX 3	Cock	,,	peas and beans	potato 5 some Labiate. Much other stem tissue	grass clover*	—	wheat: hand-fed 56 germinating 30	—	—	soil pulpy vegetable matter
	IX 4	Cock	,,	plough	? dock	scarlet pimpernel	—	—	—	—	—

* A trace. † A little. § Much.

March	IX 5	Cock	,,	,,	? dock	clover*	—	—	—	—	—
	IX 6	Cock	,,	,,	—	grass* clover*	—	barley 386‡	—	—	shot 1 stones 3
	IX 7	Cock	,,	park	—	grass*	—	—	—	—	—
	IX 8	Cock	,,	near marsh	—	clover buttercup dock	—	germinating oats 75	—	—	soil
	X 1	Cock	Croxton, Cambs.	arable, sown with wheat	—	clover buttercup daisy grass many leaves of Compositae	—	—	—	Bibio larvae 1644	moss
	X 2	Cock	,,	grass land in park	? 5	clover grass	—	—	grass 1	—	stone 1
	X 3	Hen	,,	wheat and grass	—	grass§ chickweed	buds 16	—	chickweed: capsules 20 seeds 27 false brome 6	—	—
	X 4	Hen	,,	arable, for potatoes	many fragments	grass 5	—	—	—	—	—
	XI 1	Cock	Brandon, Suffolk	grass, ling and gorse	—	stonecrop 24 celandine 1 bedstraw 1	—	maize 83‡	acorn 1 pine 14 (with part of cone)	spangle gall 1	stones
	XI 2	Cock	,,	,,	—	stonecrop§ grass† buttercup†	—	—	capsules of Carex sp 6	spangle galls 2	moss
	XI 3	Cock	,,	,,	—	grass† Galium†	Carex 50	—	Carex sp. 330	—	moss
	XI 4	Cock	,,	,,	—	—	—	maize 130‡	—	—	—
	XI 5	Cock	,,	,,	—	grass*	—	maize 114‡	—	—	some vegetable matter not identified

* A trace † A little. ‡ Hand fed. § Much

TABLE II (*continued*)

	No	Sex	District	Locality	Roots and stems	Weed leaves	Flowers	Agricultural seeds	Weed seeds	Insects	Misc.
MARCH	XI 6	Cock	Brandon, Suffolk	grass, ling and gorse	—	grass§ buttercup	gorse 6	—	rush (? sp.) 322 capsules Carex sp. 40	—	spider stones
	XI 7	Cock	,,	,,	—	buttercup grass*	—	—	—	Bibio larvae 829	moss
	XI 8	Cock	,,	,,	horsetail 10	grass dock Galium Potentilla	woodruff 138	—	rush (? sp.) 14 capsules	—	moss 33
	XII 1	Cock	Rackheath Norfolk	grass and oats	—	clover* grass*	—	maize 4‡	—	—	1 stone
	XII 2	Cock	,,	,,	fragment of root	clover† medick 2	—	—	—	weevils 2	—
	XII 3	Cock	,,	,,	oats, barley and grass	buttercup 2 hawthorn 3 many rosaceous leaves	13 flower-fragments	maize 83‡	—	spangle galls 5	—
	XII 4	Cock	?	grass and barley	horsetail stems 6	geranium 6	—	germinating oats 370	—	—	—
	XIII	Cock	Pampisford, Cambs.	light arable	—	—	—	barley 174‡ oats 4‡ wheat 1‡ maize 1‡	—	weevils 3 wireworm 1	worm cocoons 4
	XIV	Cock	,,	,,	—	—	—	maize 17‡ wheat 17‡ barley 9‡ oats 4‡	—	—	—
APRIL	XV 1	Cock	Woodton, Norfolk	cultivated land	—	buttercup§ clover† grass† Galium†	buttercup 22 woodrush 8	—	—	Bibio larvae 222 with others crushed 1 fly larva	—

* A trace. † A little. ‡ Hand-fed. § Much.

	XV 2	Cock	Shotesham, Norfolk	,,	—	buttercup clover pine needles	buttercup 60 woodrush	maize 17‡ dari 480‡ wheat 246‡	acorn fragments	spangle galls 18	—
	XV 3	Cock	Newton, Norfolk	,,	—	unidentified 1	—	maize 54‡ dari 56‡ wheat 17‡	—	—	stones 6
	XVI 1	Cock	Didlington, Norfolk	cultivated high land	—	grass§	—	—	—	click beetles 3 weevil 1 beetle larva 1	1 shot
	XVI 2	Cock	,,	light arable	—	clover and seedling sainfoin	—	—	—	—	1 stone
	XVI 3	Cock	,,	meadow near garden	horsetail stems 4	grass† clover† buttercup† dock† celandine	dandelion 10 primrose 40 buds 32	—	—	—	—
April	XVI 4	Cock	,,	arable	—	bracken 16 grass† clover† chickweed†	catkins 14 fragments	—	pine 10 much mouse-ear chickweed	—	stones 4
	XVI 5	Cock	,,	low ground near kitchen garden	bulbous buttercup tubers 25	buttercup clover† grass†	buttercup 21 dandelion 1	—	—	weevils 2	spider 1 moss
	XVII 1	—	Dullingham, Cambs	light land	—	geranium 3 grass†	—	—	chickweed 2130 capsules (about 25,600 seeds) grass*	click beetles 2 Carabid beetles 9	—
	XVII 2	—		heavy land	horsetail stems 3	clover§	—	barley 251 germinating barley 126 sainfoin seedlings 97	—	wireworms 2 Sitones 1	—
	XVII 3	—	,,	,,	—	sainfoin§ clover*	—	barley 43 germinating barley 36 sainfoin seedlings 6	—	—	shot 2

* A trace † A little ‡ Hand fed § Much

TABLE II (continued)

APRIL

No	Sex	District	Locality	Roots and stems	Weed leaves	Flowers	Agricultural seeds	Weed seeds	Insects	Misc.
XVII 4	—	Stetchworth, Cambs.	by London Road	rhizome fragments 7	grass beech 2 clover*	Compositae 4 Leguminosae (? sainfoin) 28	—	field pansy Poa†	weevils 2 not identified 1	—
XVII 5	—	,,	light land	—	grass thistle chickweed	—	—	chickweed capsules 350 Poa†	Apion 10 Sitones 5 flies 6 Carabid beetles 3 Staphylinid ,, 2 weevil grubs 3	small snails 3 wood-lice 3 spiders 5 stones
XVII 6	—	Dullingham, Cambs	ley	—	grass† others*	—	—	chickweed 80 Poa about 42,000	Aphids 28	stones spider
XVII 7	—	,,	near station	—	clover 12	—	—	chickweed 520 Poa†	weevils 6	—
XVII 8	—	Stetchworth, Cambs.	heavy land	—	clover (2 spp.) buttercup grass other fragments	dandelion 3	oats 78	—	muscid fly grubs 190 Staphylinid 6 Lamellicorn 2 wireworm 1	—
XVIII 1	Cock	Alderton, Suffolk	barley	—	grass*	gorse 46	—	chickweed spp. 240	weevil 1 other larvae 2	wood-louse small stones
XVIII 2	Cock	Bawdsey, Suffolk	,,	—	grass† Galium sheep's sorrel	inflorescences of sorrel	—	spurrey capsules 56 Poa	—	1 shot
XIX 1	Cock	Bury St Edmunds	edge of park	root fragment	—	—	—	—	—	—
XIX 2	Cock	,,	,,	—	—	—	—	—	—	—
XIX 3	Cock	Clopton, Suffolk	near barley and oats	—	grass*	—	—	—	—	—
XIX 4	Cock	,,	near wheat, barley and peas	bulbous buttercup 28	buttercup	Rosaceous flower fragments 6	—	—	Empis 1 beetle (? sp.) 1	—

* A trace. † A little.

MAY	XX 1	Hen	Elveden, Suffolk	barley	—	clover† unidentified 2	—	—	pine 3 Italian rye grass 1 Poa nemoralis	chafers (*P. horticola*) 16	—
	XX 2	Cock	,,	wood	—	clover† knapweed and other Compositae ? Silene	Silene 4 knapweed 2 other Compositae 12	—	vetch 56 acorns 2 pine 4 chickweed 500	weevil 1 Lamellicorn 1	—
	XX 3	Cock	,,	,,	—	chickweed thistle	—	sainfoin in husk 223 fragments of sainfoin seedlings	chickweed 36 pine 8	saw-fly 1 Thereva 1 ant 1 Sitones 1	1 shot
	XX 4	Cock	,,	heath	bark and decayed wood	stonecrop 225 grass Galium	Compositae fragment	—	pine 18 dove's foot geranium 6 wavy aira§	weevils 7 ichneumon 1 ant 1	spider moss
	XX 5	Cock	,,	rough ground	stem fragments	Galium sp.	—	—	pine 269	Ceropid 1 Tipula 1 muscid 1	moss
	XX 6	Cock	,,	cultivated land	—	grass§ sheep's sorrel§ Galium clover chickweed	—	—	chickweed 6 pine 3	click beetle 1	stones
	XX 7	Cock	,,	,,	—	grass†	Compositae fragments 13	—	field pansy 7000 sandwort 40 chickweed 800 capsules (=about 10,000 seeds)	dung beetles 12 saw-fly 1 ant 1	stones
	XX 8	Cock	,,	rough fen	lesser celandine 325	—	buttercup 6 Carex 60	—	—	—	soil
	XXI 1	Cock	Didlington, Norfolk	freshly sown mangolds	—	grass 6 unidentified fragments 7	—	—	Poa (annual, meadow, rough)§ chickweed 1	—	—
	XXI 2	Cock	,,	meadow	—	clover grass ? periwinkle	—	—	—	—	—

† A little § Much.

TABLE II (*continued*)

	No	Sex	District	Locality	Roots and stems	Weed leaves	Flowers	Agricultural seeds	Weed seeds	Insects	Misc.
MAY	XXI 3	Cock	Didlington, Norfolk	light arable	—	grass* Cruciferous fragments	—	—	procumbent speedwell 140 pine 3 shepherd's purse	click beetle 1 weevil 1 Telephorid 1	—
	XXI 4	Cock	,,	arable	—	—	—	—	chickweed 1210 Poa (annual, meadow, rough)§ spurrey 4	Telephorid beetles 4	—
	XXI 5	Cock	,,	park, near kitchen garden	bulbous buttercup 18	clover 8 other 3	—	—	—	click beetle 2 Staphylinid 7	—
	XXI 6	Cock	,,	low meadow	—	clover§ Galium vetch dock	buttercup 9	—	chickweed 1	—	moss 1 shot
	XXI 7	Cock	,,	,,	lesser celandine 134 horsetail stems	chickweed§ buttercup clover*	buttercup 33	—	meadow Poa†	saw fly 1	—
	XXI 8	Cock	,,	high arable	—	grass§	—	—	field pansy 30 sandwort 381 shepherd's purse (thousands)	chafers (*P. horticola*) 8 Carabids 2 fly 1	—
JUNE	XXII 1	Cock	Suffolk	grass	—	grass*	bulbous buttercup 3 Compositae 6	—	buttercup 3310 chickweed 20 Poa inflorescence fragments 120 plantain	weevil 1	—
	XXII 2	Cock	,,	mangolds	bulbous buttercup 3	clover 16	—	—	buttercup 81 meadow Poa	aphis 5 Mycetophilid larvae 9 Staphylinid 1 maggot 1 Neuropteron 1	—

* A trace † A little § Much.

JUNE	XXII 3	Cock	,,	oats	bulbous buttercup 9	—	—	—		—		—		—
	XXII 4	Cock	,,	rape	—	—	—	wheat maize	25‡ 58‡	buttercup	31	—		—
	XXII 5	Cock	,,	—	lesser celandine 377	1 leaf *	—	—		buttercup Poa	520	Telephorid Empis	1 1	—
	XXII 6	Cock	,,	—	—	—	—	wheat maize linseed hemp dari	144‡ 20‡ 7‡ 35‡ 73‡	goose grass	1	—		sand grit
	XXII 7	Cock	,,	—	—	—	—	dari wheat maize	50‡ 45‡ 1‡	—		—		—
	XXII 8	Cock	,,	rape	bulbous buttercup 5	grass†	—	hemp wheat	9‡ 4‡	buttercup	2000	earwig	1	sand grit shot
JULY	XXIII 1	Cock	Long Melford, Suffolk	wheat near barley and clover	—	2 fragments	Compositae 21	wheat	323‡	red clover	2	—		1 stone
	XXIII 2	Hen	,,	barley	—	—	—	wheat	252‡	—		aphis Cercopid bug	1 1	centipede 1
	XXIII 3	Hen	,,	mangolds	—	—	—	wheat	2‡	—		—		—
	XXIII 4	Hen	,,	beans	—	—	—	beans barley	29‡ 21‡	—		—		—
	XXIII 5	Cock	,,	clover near beans	—	grass*	—	beans	20‡	—		Sitones	7	—
	XXIII 6	Cock	,,	fallow near barley	—	—	—	barley oats	219‡ 125‡	black bindweed	2	wasp Cercopid bug fly maggot		—
	XXIII 7	Cock	,,	130 acre wood	—	—	—	maize wheat	11‡ 3‡	—		Mycetophilid larvae Staphylinid	14 1	—

* A trace. † A little ‡ Hand-fed.

TABLE II (*continued*)

July

No	Sex	District	Locality	Roots and stems	Weed leaves	Flowers	Agricultural seeds	Weed seeds	Insects	Misc
XXIII 8	Hen	Long Melford, Suffolk	barley	—	grass*	—	maize 40‡ wheat 3‡	—	Mycetophilid larvae 7 Staphylinid 2 fly 1	snail spider centipede shot 3
XXIV 1	Cock	Taverham, Norwich	clover ley near wood	—	grass*	—	awned barley 128	Italian rye grass 1180 ragged robin 84 field pansy 110 trefoil 21 scarlet pimpernel 20 field madder 3 buttercup 4 plantain 7 knot grass 1	aphis 1 weevil 1 larva of water insect 1	1 shot 1 stone
XXIV 2	Cock	,,	bracken near fir covert	—	—	—	awned barley 38	bindweed 400 sandwort 140 chickweed 25 scarlet pimpernel 53	—	—
XXIV 3	Cock	,,	,,	—	fragments (? sp.)	? dandelion 37	barley 3	bindweed 1540 sandwort 320 pimpernel 70 buttercup 1 plantain 1	—	—
XXIV 4	Cock	,,	meadow	bulbous buttercup 7	buttercup	buttercup 39	—	buttercup 9000 Yorkshire fog 65 Glyceria 46 chickweed 10 plantain 1	Capsids 2 ant 1 undetermined larvae 3	centipede spiders 2
XXIV 5	Cock	,,	rough grass	—	—	—	wheat 92‡ dari 12‡ maize 7‡	Carex sp. 100 tall oat grass 56 rough stalked meadow grass 65 Yorkshire fog 46 sheep's fescue 8 buttercup 1	3 ants and 1 pupa	1 shot

* A trace ‡ Hand fed

July	XXIV 6	Cock	Attlebridge, Norfolk	oats	—	—	—	oat 640	—	thrips 18 aphis 18 Sitones 2 ladybird larvae 2 others 5	stone 1 spider
	XXIV 7	Cock	Taverham, Norwich	marshy meadow	—	clover 20 Galium (sp.) 1	butter-cup 192	—	buttercup 2300 meadow fescue 840 ragged robin 22 Carex sp. 40 celery leaved buttercup 1170	Mycetophilid grubs 138 wasps 2 ichneumon and flea beetle 1	—
	XXIV 8	Cock	,,	,,	—	? 2 fragments	butter cup 108	—	buttercup 1000 fescue 1110 Glyceria 25 ragged robin 4	Mycetophilid grubs 564 Therevid fly 2 fly maggots 2	soil
	XXV 1	Cock	Hindolve-stone, Norfolk	swedes	—	15 fragments unidentified	—	—	rough stalked meadow grass+	aphis 4 thrips and springtail	—
	XXV 2	Cock	Swanton Novers, Norfolk	wheat	—	—	—	—	soft brome 51 chickweed 56 spurrey 122 rush (sp.) 42 silene pimpernel meadow grass	aphis 25 thrips 3 ant 1 springtail	—
	XXV 3	Cock	Briningham, Norfolk.	swedes	bulbous buttercup 6	—	—	—	acorn buttercup 15 meadow grass	Cercopid 1 Psocid 1	—
	XXV 4	Cock	Wood Norton, Norfolk	barley	—	wild clover 60 clover 3	Compositae 4	wheat 3	rye grass 8	Cercopid 1	Annelid 2 fragments
	XXV 5	Cock	Melton Constable, Norfolk	wheat	—	—	—	wheat 100	decumbent Triodia 312 charlock 2 oat and knot grass 1	—	stones 2
	XXV 6	Cock	Briston Norfolk		—	poppy 2	poppy 2 buds	wheat 281	1 capsule (Caryophyllaceae)	—	—

+ A little

TABLE 11 (*continued*)

August

No.	Sex	District	Locality	Roots and stems	Weed leaves	Flowers	Agricultural seeds	Weed seeds	Insects	Misc.
XXVI 1	Cock	Riddlesworth, Norfolk	light arable	—	? Silene	—	oats 636	Silene 4 bindweed 1 enchanter's nightshade 1	Formica fusca 3 chafer 1	—
XXVI 2	Cock	Gasthorpe, Norfolk	arable	—	—	—	wheat 224	buttercup 5 soft brome 1 rye grass 2	ants (2 spp.) 1183 and 150 pupae mites 18	—
XXVI 3	Cock	,,	light arable	—	fragments ?	1 bud	awned barley 627	bindweed 155	Sitones 3 Asilus 3 Neuropteron	stones 3
XXVI 4	Cock	Riddlesworth	pasture near river	horsetail stem 3	buttercup 88 grass clover	sainfoin 1	sainfoin in husk 47	Yorkshire fog 620 plantain 1	ants 8 gnat 1 mangled water insects	1 shot
XXVI 5	Cock	Gasthorpe	arable	—	clover§ grass buttercup	buttercup	—	rye grass 3000 Yorkshire fog 300 toad flax 15 white clover 6 plantain 4 chickweed 2	ants 1 Tipula 1 other 3	stones 14
XXVI 6	Cock	Riddlesworth	pasture near river		—	—	wheat 98	—	ants 3 beetle 1	—
XXVII 1	—	Orwell Park	arable near stubble	bulbous buttercup 32	clover*	Compositae 2	wheat 40	rye grass 1 chickweed 2	—	—
XXVII 2	—	,,	,,	—	10 fragments	—	wheat 363	spurrey 28 sorrel 2	—	1 shot
XXVII 3	—	,,	grass	—	—	—	—	—	—	—
XXVII 4	—	,,	arable	—	—	—	rye 43	—	—	—
XXVII 5	—	,,	heath near stubble	—	—	—	rye 125	—	—	1 stone

* A trace. § Much.

AUGUST	XXVII 5	—	„	grass near stubble	—	blackberry		—		peas	7	blackberries	5	weevils Carabid fly	2 1 1	wood louse stone
	XXVII 7	—	„	„	—	poppy clover ? convolvulus	12 3	poppy Compositae?	2	wheat barley	19 2	bindweed sandwort clover (yellow suckling) rye grass blackberries	123 240 30 12 7	Carabids Thereva larvae Capsid ladybirds caterpillar ichneumon	22 2 1 4 1 1	spider
	XXVII 8	—	„	mangolds	—	11 fragments		Compositae 3		—		field madder	3	weevil ichneumon	1 1	—
	XXVIII 1	Cock	Stow, Downham Market	near station	—	grass		—		oats	35	privet Rubus sp	14 13	—		stones 72
	XXVIII 2	Hen	„	„	—	—		buttercup	2	black oats	56	chickweed	1	surface cater-pillar Tipul	1 8	1 shot stones 147
SEPTEMBER	XXIX 1	Hen	Wickham brook, Suffolk	seeds and stubble	—	sainfoin*		—		wheat	51	soft brome rye grass	3 4	stones	1	spider wood-louse centipede
	XXIX 2	Cock	Owsden	sainfoin	—	blackberries	20	—		wheat oats	43 27	blackberries vetch knapweed	7 5 3	Tipula	1	—
	XXIX 3	Cock	Ashley	stubble	—	clover	30	—		wheat barley	602 7	blackberries vetch	2 2	Tipula	1	snail, spider soil, shot
	XXIX 4	Cock	„	roots	—	clover*		—		barley wheat	6 1	blackberries other seeds	10 40	Tipula weevils others	19 2 2	2 snails 1 spider
	XXIX 5	Hen	Owsden	sainfoin stubble	—	clover* grass*		—		oats awned barley	77 24	blackberries herb bennet chervil knapweed	11 32 6 3	ladybird	1	earth-worm 1
	XXIX 6	Hen	„	stubble	—	grass sainfoin		—		oats barley wheat	551 64 16	—		—		1 snail

* A trace.

TABLE II (*continued*)

	No	Sex	District	Locality	Roots and stems	Weed leaves		Flowers	Agricultural seeds		Weed seeds		Insects		Misc
SEPTEMBER	XXIX 7	Hen	Owsden	grass	—	—		—	wheat	9	blackberries	3	—		1 snail
	XXIX 8	Cock	„	stubble	—	grass clover*	22	—	wheat	66	—		—		—
	XXX 1	Cock	Gt Glemham, Suffolk	cultivated land ¼ mile from feed	—	thistle	6	—	barley oats	105 1	plantain other	12 4	stones	2	1 shot
	XXX 2	Cock	Parham, Suffolk	„	—	—		—	wheat peas	406 10	Italian rye grass	4	—		stones
	XXX 3	Cock	Marlesford, Suffolk	„	—	grass*		—	awned barley	56‡	bindweed	6	ant Capsid	1 1	wood-lice17
	XXX 4	Cock	„	„	—	—		—	barley	314‡	—		—		1 stone
	XXX 5	Cock	Ash, Suffolk	„	—	4 fragments*		—	barley wheat	273‡ 4‡	—		ant	1	1 shot
	XXX 6	Cock	Gt Glemham	„	—	5 fragments*		—	wheat peas	115 13	—		—		—
	XXX 7	Hen	Parham	„	—	—		—	oats barley	357 1	poppy capsule		—		—
	XXX 8	Hen	Ash	„	—	—		—	wheat	195	bindweed	2	—		1 stone
OCTOBER	XXXI 1	Hen	Littleport, Cambs	fen	—	—		—	wheat oats millet dari	41‡ 1‡ 12‡ 1‡	bindweed	1	—		—
	XXXI 2	Cock	„	„	? fragments	grass	20	—	—		bindweed fat hen hemp nettle chickweed Persicaria	29 45 6 4 1	—		vegetable pulp

* A trace ‡ Hand fed

October	XXXI 3	Cock	,,	plantation where food is laid	—	grass	—	wheat 377 canary grass 10 hemp 5 maize 3 oats 3‡	chickweed 340	—	—
	XXXI 4	Hen	,,	arable, wheat, oats and hemp	—	—	—	maize 25‡ wheat 8‡ oats 1‡ millet 1‡	Rubus sp 5 chickweed 5 other 12	—	—
	XXXII 1	—	Littleport, Cambs	rough grass	—	—	—	oats 1	chickweed 90	—	—
	XXXII 2	—	,,	,,	—	? 5 fragments	—	—	chickweed 660	Eristalis sp 27	—
	XXXII 3	—	,,	rye	—	? 7 fragments	—	germinating rye 144	field pansy 175 chickweed 150 bindweed 130 Persicaria 1	aphis 2 muscid flies 2 Capsids 2	centipede
	XXXII 4	—	,,	,,	root fragments 18	—	—	germinating rye 178	Carex sp 404 hemp 2 knapweed 1	—	—
	XXXIII 1	—	Sprowston Norfolk	spinney near stubble	—	clover 11	—	awned barley 288	bindweed 14	spangle gall 1	—
	XXXIII 2	—	,,		—	—	—	—	acorns 3	—	—
	XXXIII 3	—	,,	plantation near arable and barley stubble	—	grass*	—	barley 165	—	spangle galls 3 surface caterpillars 4 other 4	—
	XXXIII 4	—	,,	,,	—	grass clover 20	Compositae 34	—	acorns 12	spangle galls 72	moss
	XXXIII 5	—	,,	,,	—	—	—	—	acorns 8	spangle galls 12	18 shot
	XXXIII 6	—	,,	,,	—	—	—	—	acorns 5 field pansy 60 knot grass 60 fat hen 10 meadow fescue 1	—	—

* A trace ‡ Hand-fed.

TABLE II (*continued*)

	No.	Sex	District	Locality	Roots and stems	Weed leaves	Flowers	Agricultural seeds	Weed seeds	Insects	Misc.
OCTOBER	XXXIII 7	Hen	Sprowston, Norfolk	plantation near park	—	—	—	—	acorns 3 Carex sp. many	spangle galls 14 beetle 1	—
	XXXIII 8	Hen	,,	,,	—	—	—	—	Carex sp. many	—	—
	XXXIII 9	Hen	,,	woods near wheat and arable	—	grass 2 fern 2 ? 1	—	barley 738	bindweed 355 pine 2 lesser bindweed 2 greaterknapweed 2	spangle galls 8	1 stone
	XXXIII 10	Cock	,,	,,	—	pine needles grass fern	—	barley 1275	lesser bindweed 2 black bindweed 1 field scabious 2 Persicaria 1 thistle 1	—	—
NOVEMBER	XXXIV 1	Hen	Hilgay	long grass fen	—	—	—	—	Carex (sp.)§	—	—
	XXXIV 2	Hen	,,	osiers fen	—	clover† dock† speedwell†	—	rye 86	speedwell 6 acorns 2 other 6	spangle galls 457 other 2	snails 3 moss
	XXXIV 3	Hen	,,	ash wood skirt land	—	—	—	maize 1‡	acorns 3 knapweed 1	spangle galls 3	soil 1 shot
	XXXIV 4	Cock	,,	kale	—	—	—	—	knotgrass 5 hemp nettle 3 Persicaria 9680 chickweed 60 Silene 7 bindweed 132 fat hen 15	spangle galls 2	—
	XXXIV 5	Hen	,,	swedes	—	daisy 2 clover 16 grass*	—	maize 1‡ barley 32 germinating barley 2	blackbindweed 400 mayweed 322 fat hen 165 chickweed 33 privet 8	—	—

* A trace † A little. ‡ Hand fed. § Much.

November	XXXIV 6	Hen	,,	grass near fir wood	—	clover grass	14	—	—	barren brome 80 bindweed 2 Persicaria 1	spangle galls 2	—
	XXXIV 7	Cock	Downham Market	arable	—	grass		—	awned barley 17	chickweed 30 acorns 31 plantain 4 fat hen 3 knot grass 2	spangle galls 9	soil stones shot 2
	XXXIV 8	Cock	,,	grass	—	—		—	maize 18‡	—	—	—
December	XXXV 1	—	Drinkstone park	woods	—	—		—	—	acorns 6	—	—
	XXXV 2	—	,,	,,	—	—		—	—	acorn	—	—
	XXXV 3	—	,,	,,	—	—		—	maize 1‡	acorns 3	—	—
	XXXV 4	—	,,	,,	—	grass§		—	—	acorns 14 seeds (?damson) 5	—	—
	XXXV 5	—	,,	pasture near wheat	—	—		—	—	acorns 15 plantain 1	—	—
	XXXV 6	—	,,	,,	—	grass scarlet pimpernel other fragments	16	—	—	knot grass 1080 fat hen 1328 chickweed 84 plantain 25 bindweed 15 speedwell 5 meadow grass§ black nightshade 9	Carabid 1 ichneumon 1	sand snails 2 stone
	XXXV 7	—	,,	,,	—	—		—	—	acorns 15	—	—
	XXXV 8	—	,,	,,	—	grass*		—	—	fat hen 1328 knot grass 1080 meadow grass§ speedwell 5 chickweed 84 plantain 25 nightshade 8	Carabid 1 ichneumon 1	sand snails 2 stone

* A trace. ‡ Hand-fed. § Much.

TABLE II (*continued*)

	No.	Sex	District	Locality	Roots and stems	Weed leaves	Flowers	Agricultural seeds	Weed seeds	Insects	Misc
DECEMBER	XXXVI 1	—	Norfolk	—	bulbous buttercup 3	—	—	barley 35‡ germinating oats 1	acorn 1	—	—
	XXXVI 2	—	,,	—	—	grass* clover*	—	—	acorns 2	spangle gall 1	—
	XXXVI 3	—	,,	—	—	grass§ clover§	—	—	plantain 40 meadow grass 3 acorn 1 ash 1	spangle gall 1 maggot 1	earthworm
	XXXVI 4	—	,,	—	—	—	—	—	acorns 2	—	—
	XXXVI 5	—	,,	—	—	—	—	barley 2‡	—	—	—
	XXXVI 6	—	,,	—	bulbous buttercup 16	—	—	—	pine 5 silver birch 1	—	—
	XXXVI 7	—	,,	—	bulbous buttercup 2	—	—	—	acorns 4	spangle galls 5	—
	XXXVI 8	—	,,	—	bulbous buttercup 8	—	—	—	acorns 4 plantain 5	spangle galls 38	—

* A trace ‡ Hand fed § Much

TABLE III.

	No	Date when shot	Agricultural seeds non-hand-fed	Remarks
JANUARY	I 9	Jan. 13	germinating barley 1	Probably tail barley which had begun to germinate. Crop nearly empty.
	III A	Jan. 16	**germinating wheat 64**	The mixed contents of 40 crops from fen land, cultivated land near wheat, beans and cole seeds. Much soil in bottle.
	VI 1	Jan. 21	**germinating wheat 11** other wheat 35	The germinating grains were covered with soil; no hand-fed grain given. Arable land.
	VI 2 and 3	,,	**germinating wheat 33**	do. do. do.
	VII	Jan. 26	peas 12	The mixed contents of 8 crops, and therefore insignificant.
FEBRUARY	VIII 3	—	maize 118	No food given. Must have picked up near some house or farm.
	VIII 4	—	germinating beans 6	do. do. do.
	VIII 5	—	maize 2	do. do. do.
	VIII 6	—	germinating beans 25	Fen soil, Fordham; no note of bean fields near.
MARCH	IX 1	—	germinating wheat 1	Probably hand-fed grain which had sprouted.
	IX 3	—	**germinating wheat 30**	Shot in bean field near new sown wheat.
	IX 7	—	**germinating oats 75**	Shot in oats and ley. Soil in crop.
	XII 4	March 30	**germinating oats 370**	In grass and barley but near oat field.
APRIL	XVII 2	April 27	**germinating barley 126** **other barley 251** **sainfoin seedlings 97**	Heavy land at Dullingham; barley and seeds near.
	XVII 3	,,	**germinating barley 43** **other barley 36** **sainfoin seedlings 6**	do. do. do.
	XVII 8	April 29	oats 78	Grass land. No soil in crop. Other food, buttercup, clover, grass, insects. Probably picked up near stack.
MAY	XX 3	May 26	**sainfoin in husk 223** fragments of sainfoin seedlings	Wood at Elveden. Wheat and barley hand-fed. Natural food scarce. Other food in crop, pine seeds, weed leaves, insects.
JUNE	none			

TABLE III (*continued*)

	No	Date when shot	Agricultural seeds non-hand-fed	Remarks
July	XXIII 4	July 21	beans 29 barley 24	Bean field. No other food in crop. Probably bean-pods had split, July 1914 being hot.
	XXIII 5	July 22	beans 20	Clover near bean field. Other food, 7 weevils.
	XXIII 6	July 24	oats 125 barley 219	Fallow, near barley.
	XXIV 1	July 15	awned barley 128	Clover ley near wood. Probably picked up from a few broken-off ears lying about.
	XXIV 2	,,	awned barley 38	do. do. do.
	XXIV 3	,,	barley 3	Bracken. Probably a few ears lying about.
	XXIV 6	July 23	oats 640	Oat field. Other food, insects. Harvest probably beginning.
	XXV 4	July 21	wheat 3	
	XXV 5	July 21	wheat 100	Wheat field. Not known if any hand-fed food given.
	XXV 6	July 29	wheat 281	do. do. do.
August	XXVI 1	Aug. 10	oats 636	Light soil. Probably from stubble
	XXVI 2	,,	wheat 221	Probably from stubble.
	XXVI 3	Aug. 15	awned barley 627	do. do.
	XXVI 4	Aug. 17	sainfoin in husk	Pasture near river.
	XXVI 6	Aug. 28	wheat 98	Probably from stubble.
	XXVII 1	Aug. 15	wheat 10	Shot near stubble.
	XXVII 2	Aug. 17	wheat 363	do. do.
	XXVII 4	Aug. 19	rye 13	do. do.
	XXVII 5	Aug. 25	rye 125	do. do.
	XXVII 6	,,	peas 7	Shot in grass near stubble.
	XXVII 7	Aug. 28	wheat 19 barley 2	do. do.
	XXVIII 1	Aug. 29	oats 35	Near Stow station. Probably from stubble or stack yards.
	XXVIII 2	,,	black oats 56	do. do. do.
September	XXIX 2	Sept. 7	oats 27	Shot in sainfoin. Oats probably from stubble.
	XXIX 3	Sept. 11	barley 7	Shot in stubble.
	XXIX 4	Sept. 14	barley 6	Shot in roots. Barley from stubble

TABLE III (*continued*)

Month	No.	Date when shot	Agricultural seeds non hand fed	Remarks
SEPTEMBER	XXIX 5	Sept. 17	oats 77 awned barley 24	Shot in sainfoin. Grain from stubble.
	XXIX 6	Sept. 18	oats 551 barley 61	Shot in stubble.
	XXX 1	Sept. 9	oats 1	
	XXX 2	Sept. 10	wheat 406 peas 10	Probably picked up in stubble.
	XXX 5	Sept. 18	wheat 4	do. do. do.
	XXX 6	,,	wheat 115 peas 13	do. do. do.
	XXX 7	Sept. 19	oats 357	do. do. do.
	XXX 8	,,	wheat 195	do. do. do.
OCTOBER	XXXI 3	Oct. 13	hemp 5 oats 3	Shot near plantation where food is laid. Arable is wheat, oats and hemp. From stubble.
	XXXI 4	,,	oats 1	do. do. do
	XXXII 1	Oct. 16	oats 4	Rough grass. Probably from stubble.
	XXXII 3	,,	**germinating rye 144**	Shot near rye field.
	XXXII 4	,,	**germinating rye 178**	do. do.
	XXXIII 1	Oct. 8	awned barley 288	Shot near barley stubble.
	XXXIII 3	Oct. 15	barley 165	do. do.
	XXXIII 9	Oct. 29	barley 736	Shot in wood near new sown wheat. Probably from stubble.
	XXXIII 10	,,	barley 1275	do. do.
NOVEMBER	XXXIV 2	Nov. 12	rye 86	Shot among osiers. Fen soil near cultivated land.
	XXXIV 5	Nov. 19	barley 32 germinating barley 2	Shot on fence dividing swedes. No food near. Perhaps near stack yard.
	XXXIV 7	Nov. 28	awned barley 17	
DEC.	XXXVI 1	Dec. 29	germinating oats 1	Shot in wheat.

(*Received January 17th*, 1918.)

THE INFLUENCE OF PLANT RESIDUES ON NITROGEN FIXATION AND ON LOSSES OF NITRATE IN THE SOIL.

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(With Three Figures in text.)

THE incorporation of plant residues with the soil may give rise to a number of different changes, any one of which tends to dominate the rest according to the varying conditions of the general environment—air and water supply, temperature, the reaction of the soil itself. Whilst, under average conditions the easily decomposable constituents of the plant are speedily resolved by the action of the soil flora, the more resistant structures often persist unchanged for considerable periods, but ultimately lose their identity and as humus become an integral part of the soil. The generally accepted statement that organic matter possesses value on account of its humus forming properties does not express fully the influence of organic residues, and it is quite probable that the primary and intermediate stages of decomposition are equally important from the standpoint of soil fertility.

An indication of such effects of plant residues is afforded by two of the Rothamsted plots which, since 1882 and 1885, have been allowed to revert from arable to prairie conditions. As the result of analyses of the soil of these plots, Hall (1) was able to show that during the twenty year period of the observations very considerable accumulations of nitrogen had taken place, and although it was recognised that the conditions were complicated by the presence of leguminous plants in the herbage of one of the plots, by the absorption of free ammonia from the atmosphere, and the possible capillary uplift of nitrates from the permanent soil water it was, at the same time, considered that part of these gains might reasonably be ascribed to the activity of certain nitrogen fixing organisms which have been found in these soils by Ashby (2). It was, in fact, considered that the carbohydrates formed by the decomposition of the crop

residues might to some extent subserve the process of nitrogen fixation. This view accords with the conclusions formed by Henry (3), who was able to demonstrate that when leaves are kept in a moist but aerated condition a slight assimilation of atmospheric nitrogen takes place. This was in the first instance disputed, but subsequently confirmed, by Hornberger (4).

The experiments discussed in the following pages were originally undertaken with the object of ascertaining in the first place whether and in what manner these residues become available for the assimilation of atmospheric nitrogen, and how far the conditions in the field are favourable for the operation of the process to any appreciable extent.

Since the discovery of such nitrogen fixing organisms as *Clostridium Pastorianum* by Winogradsky (5) in 1893, and more particularly *Azotobacter chroococcum* by Beijerinck (6) in 1901, a considerable amount of work has been carried out, but chiefly with reference to the cultivation of these organisms under laboratory conditions and with synthetic media. Such work as that of Freudenreich (7), Gerlach and Vogel (8), Remy (9), Löhnis and his scholars (10), Heinze (11), Lipman (12), Haselhoff and Bredemann (13), Warmbold (14), Stoklasa (15), Schneider (16) and many others sufficed to show not only that these bacteria are widely distributed, but that under certain conditions of temperature, aeration and food supply, an energetic assimilation may be induced.

Much of this work, though valuable from a physiological standpoint, was somewhat lacking in general applicability, and it is mainly due to the investigations of Koch and his collaborators (17) that we possess some conception of the intensity of the process under more natural conditions in the soil itself.

In these experiments the addition of sugar to the soil resulted in gains of 3–10 mgrms. of nitrogen per gram of carbohydrate supplied, but later work showed that when calculated on the amounts of sugar actually oxidised these increments were of a much higher order. The best utilisation of the sugar occurred when dextrose was applied at the rate of 2·0 per cent. of the weight of the soil, or when 0·2 per cent. was added eight times, or 0·5 per cent. five or eight times. A limit to the efficiency of these repeated applications was however soon reached, and the interesting observation was made that when the lower proportions were more frequently used or when the higher doses such as 1·0, 1·5 or 2·0 per cent. were applied five times, a proportionate decrease in activity ensued. Part of this effect was, no doubt, due to the high concentration of sugar in the soil water, but it may also be attributed, with a fair degree of

probability, to the inimical effect of an ever increasing degradation of the nitrogen compounds already formed by the bacteria and a consequent accumulation of available nitrogen compounds.

Definite fixation was also obtained by the use of saccharose, but the addition of molasses to the soil resulted in a loss during six weeks to the extent of 30 per cent. of the nitrogen originally present. This was attributed, though without any definite experimental evidence, to the liberation of free nitrogen from the easily decomposable nitrogen compounds in the molasses and to the above mentioned suspension of nitrogen fixation in the presence of such bodies.

Filter paper and buckwheat straw were without apparent effects when incorporated with the soil, but mustard caused appreciable losses under similar conditions.

From numerous pot experiments with oats, buckwheat and sugar beet it was evident that the nitrogen stored up by bacterial action readily becomes available for plant growth, and that by the treatment of the soil with sugar, its fertility may be increased to a marked degree.

These results have been fully confirmed by Remy (18) who was also able to demonstrate the intimate relationship existing between the supply of magnesia, lime, and mineral manures, and a rich and potentially virulent population of Azotobacter in field soils.

The possibility of reproducing on a field scale the conditions obtaining in the above experiments appears at first glance to be somewhat remote, but attention is drawn by Ebbels (19), to the fact that molasses has for a long time been looked upon as a valuable fertiliser for sugar-cane lands in Mauritius. As he was in possession of a large quantity of molasses which could not be disposed of in any other manner a portion of this material was applied to field soil which subsequently was planted with sugar-cane. The soil so treated was found to be not only more productive than that which had not received molasses, but showed five years later a much higher nitrogen content than the control. These results have been corroborated by Boname (20) who obtained considerable increases of crop, especially in those cases where phosphatic manures were applied in addition to molasses. The effect of treatment was greatest with the first crop, but was found to persist up to the third ratoons. Experiments in the Leeward Islands (21) gave, in three cases out of four, increases varying from 1·8 to 7·7 tons of sugar cane per acre, and in this case also a residual effect was observed. Similar work has also been carried out in Java (22).

On the other hand, numerous instances are on record, of which those

described by Harrison and Ward (23) are typical, where the application of saccharine material has been quite ineffective in promoting greater fertility.

In many of these cases it is difficult to account for the absence of any specific effect of the treatment on crop yield, owing to the lack of information as to the precise conditions under which the experiments were carried out. The possibility is not excluded, however, that this ineffectiveness is the resultant of two distinct sets of changes, namely, the beneficial effects which have been discussed above, and the distinctly injurious ones which have been observed by Hiltner (24), von Seelhorst (25), Stützer (26), Bartels (27) and others to result from the admixture of straw, green manures and so forth, with the soil. The tendency towards divergent results has been recognised by Peck (28) who states that harmful effects are likely to attend the application of molasses at frequent intervals to growing sugar cane, but that when applications are made some weeks prior to the introduction of a crop, beneficial results may be obtained.

The experiments described in the following pages afford additional evidence of each of these effects. The incorporation of such substances as sugar, starch, or plant residues with the soil is likely to give rise to two opposite processes, one of which results in a diminution, and the other in an increase of the fertility of the soil. Whether the one preponderates over the other, or an equilibrium is attained, depends largely on the quantity and type of material applied, the prevailing temperature, the interval before the introduction of a crop, and the presence of the specific micro-organisms.

In any case the destructive changes come first into operation but their relative intensity and the period of persistence are increased by low temperatures. High temperatures by permitting of the entrance and subsequent predominance of constructive bacterial changes, tend to limit the extent and persistence of soil losses.

The experiments show that the introduction of a crop immediately after the incorporation of material containing carbohydrates will result in decreased yields. If a long period is allowed to elapse before the crop is sown this decrease may be only slight or entirely absent in spite of low temperatures. If the soil temperature is high and sufficient time is given before a crop is sown, adverse changes are likely to be reduced to a minimum and favourable after-effects may be anticipated.

The autumn application of plant residues, strawy material, etc., may therefore be expected to lead to beneficial changes; spring applications

of such material in any quantity are inadvisable unless the constituents likely to give rise to adverse changes can be previously eliminated. This possibility forms the subject of a separate inquiry which is proceeding at the present time.

It is apparent that the question possesses something more than purely academic interest, especially in its relation to the present day tendency towards a decreased production of manure and an increased output of straw and similar materials. It is also linked up more or less directly with a number of minor issues such as the utilisation of green manures, the preparation of compost, and of leaf and turf mould. At the same time it is unfortunately a field in which little more than sporadic investigation has yet been attempted, and where consequently practice not infrequently follows a purely arbitrary course.

EXPERIMENTAL.

A. *Field Experiments on Nitrogen Fixation.*

In order to ascertain the extent to which nitrogen fixation processes come into operation in the field, the halves of two of the Hoos Field plots which had been under continuous barley experiments since 1852, were used for this work. Of these two plots, one (6. I.) had not received any manurial dressing since the above year, while the other (4. O.) had received annually a complete mineral manure without nitrogen. The experiment was continued from 1906 to 1911, an application of one ton of sugar per acre being given each year with the exception of 1907, when potato starch was applied at the same rate. The immediate effect of the application of sugar in 1906 was a practical extinction of the barley crop, but owing to the proximity of the sugar application to the sowing of the crop it was thought that this effect might have been due to the primary decomposition products of the carbohydrate. During the next three years, therefore, a longer interval was allowed to elapse between the time of treatment and the introduction of the crop, but although the injurious effect was not so marked it was nevertheless evident in all the cases where the sugar was applied in spring. Examinations of the soil showed that the treatment had resulted in a very great increase of bacteria, and it was presumed, for the time being, that these were acting prejudicially on the crop either by the decomposition of the soil nitrates with the liberation of free nitrogen, or by the assimilation and retention of available nitrogen compounds.

A second factor which demanded consideration was the low soil temperature prevailing at the time of these earlier applications, and since other observations had shown the great differences in nitrogen fixation which occur at various temperatures up to 30°, it was decided to make the next application of sugar with a high prevailing soil temperature, that is, in early autumn.

This change was fully justified by the yields of the 1910 crop, an appreciable increase being shown by the plots which had been treated with sugar and had received an application of phosphatic and potassic manures. A second autumnal application was therefore made in 1910, and the 1911 results were of a still more promising order. (Table I and Fig. 1.)

TABLE I.

The Influence of Carbohydrates on the Fertility of Field Soils.

Hoos Field Experiments: Crop Barley: Produce per acre.

Year	Time of application	Carbohydrate supplied, 1 ton per acre	Mean soil temperature during month following application	Plot 6 I. (Unmanured 1852–1911) Grain, lbs.	Straw, lbs.	Total produce, lbs.	Relative yields	Plot 4 O. (complete minerals, no nitrogen), 1852–1911 Grain, lbs.	Straw, lbs.	Total produce, lbs.	Relative yields
1906	March 6th	None		866	631	1497	100	1209	1276	2485	100
		Sugar	4° C	—	—	—	—*	—	—	—	—*
1907	Jan. 17th	None		534	1070	1604	100	858	2720	3578	100
		Starch	−2° C	400	792	1192	74	809	2440	3249	91
1908	Feb. 4th	None		560	508	1068	100	812	1008	1820	100
		Sugar	5° C.	301	347	648	61	538	866	1404	77
1909	Feb. 19th	None		836	693	1529	100	1289	1859	3148	100
		Sugar	0° C.	542	810	1352	88	897	1364	2261	71
1910	Sept. 27th, 1909	None		625	1008	1633	100	850	1232	2082	100
		Sugar	11° C.	689	868	1557	95	1215	1287	2502	120
1911	Sept. 22nd, 1910	None		207	671	968	100	372	872	1244	100
		Sugar	12° C.	303	722	1025	105	677	1238	1915	154

* Crop failed completely.

Two interesting points arise from these results In the first place, it may be noted that although the total yields of plot 4 O. are distinctly increased as a result of treatment with sugar, this is due to a great extent to the effect of the treatment on the yield of grain rather than on that of the straw, the mean relative yields of the former being 155 per cent. as against 120 for the latter.

Secondly, it is noteworthy that in both 1910 and 1911 the application of sugar to plot 6. I., which did not receive mineral manures, was quite ineffective in bringing about any crop response. Experience has shown

that although barley grown on this soil responds readily to an application of phosphatic manures alone, it nevertheless does so to an equal or greater extent when a simple nitrogenous dressing is given. It is possible therefore that the growth of the crop on 6. I. was not limited primarily by the lack of phosphates, but by the absence of any appreciable fixation of nitrogen. This is perfectly compatible with other work, and amongst others Koch has shown the great importance of an adequate supply of available phosphates for the assimilation of nitrogen by free living organisms, as is shown by the following table.

	Soil alone	Soil + 0 1 % superphosphate	Soil + 0·6 % basic slag	Soil + 1·0 % basic slag
After 4 weeks	11 2 mgrm.	19·2 mgrm.	16 7 mgrm.	16 9 mgrm.
After 7 weeks	25 5 "	33 8 "	27·0 "	28 2 "

Finally, it should be recognised that the soil of plot 6. I., which failed to give any definite return on treatment with carbohydrates, had been persistently starved for more than fifty years, and it must not be concluded that equally unfavourable results would be obtained with any normal soil.

The experiments carried out by Boname, to which reference has already been made, indicate the expediency of ensuring an adequate supply of phosphates if an economic utilisation of the carbonaceous material is to be expected. The same also applies of course to basic compounds where the soil is naturally deficient in these constituents (29).

B. *The Utilisation of Plant Residues for Nitrogen Fixation.*

Laboratory Experiments. The organic materials returned to field soils are, in the main, the leaves of root crops, the stubble of cereal crops, green manures, and the constituents of farmyard manure. In nature, a similar position is taken by the remains of annual plants and the leaves of perennials. A number of preliminary laboratory experiments were therefore carried out to ascertain whether these substances could be used for the assimilation of nitrogen.

The chief disadvantages inherent in such laboratory work are that, in order to limit experimental error in the determination of nitrogen, the amount of soil must be kept as low as practicable, whilst in order to get appreciable quantities of nitrogen assimilated, the source of energy must be present in relatively high proportions. Hence in the case of soluble compounds the concentration of the solution soon passes the optimum for the process; in the case of plant residues, the decomposition is liable to be strictly localised and the acids initially set up by bacterial

or fungal action are only tardily removed by any base that may be present.

The gains of nitrogen shown below, although not large, are fairly satisfactory in view of the restricted conditions under which they were obtained. The substances to be tested were previously dried and finely ground and then added in quantities of 0·5 to 1·0 grm. to 10 grm. of fine air-dry soil. These portions were placed in Erlenmeyer flasks and incubated at 30° C. after sufficient water had been added to make the mixture nicely saturated.

The addition of straw at the rate of 5 per cent. gave the following results.

	Total nitrogen			
	At beginning	After 30 days	After 44 days	After 240 days
0 5 grm. straw + 10 grm. soil	15 92 mgrm.	17 50 mgrm	17·71 mgrm.	18 23 mgrm.
Gain (calculated per grm straw)	—	3·16 „	3 58 „	4 62 „

Finely ground wheat stubble and elm leaves added at the rate of 10 per cent. gave relatively lower gains.

	Total nitrogen		
	At beginning	After 15 days	After 50 days
1 grm wheat stubble + 10 grms soil	19 21 mgrm	20·09 mgrm	20 82 mgrm.
Gain	—	0·88 „	1 61 „
1 grm elm leaves + 10 grms. soil ...	33 89 „	38 18 „	37·37 „
Gain	—	4 29 „	3 48 „

The use of sand and of calcium carbonate, in conjunction with soil, gives better results, possibly on account of better aeration and a more efficient neutralisation of acid products.

	Total nitrogen	
	At beginning	After 69 days
1 grm straw + 5 grms sand + 5 grms. soil + 1 grm. $CaCO_3$	15 30 mgrm.	21·57 mgrm.
Gain	—	6 27 „

In comparison with sugar, these plant materials appear to become available for nitrogen fixation much less rapidly, but the final gains agree well with those frequently obtained when sugar is subjected to the action of a mixed bacterial flora.

Pot Experiments. With the object of obtaining further information as to the value of plant residues and sugar for the fixation of nitrogen, a number of pot experiments have been carried out, the effect of the applications being gauged in the first instance by plant growth, and at the close of the experiments by nitrogen determinations. With sugar, starch, and other non-nitrogenous materials, demonstration of their use

for nitrogen assimilation is quite simple, but in the case of plant residues some of the effects of the treatment may be due to the nitrogenous compounds contained in them apart from any nitrogen assimilated by the action of soil organisms.

Since it is impossible to make any accurate allowance for the nitrogen originally contained in the residues and which becomes slowly available for plant growth, the experiment resolves itself into a comparison between the effects of a mixed bacterial flora without nitrogen fixing organisms, and such a flora with the latter organisms present. It becomes necessary, therefore, to start with a sterile medium and for this purpose sand, and not soil, must be chosen.

The work was carried out in sixteen glazed earthenware pots, each containing 3,500 grms. of clean sand to which 2·0 per cent. of calcium carbonate, 0·1 grm. potassium phosphate, and 0·04 grm. magnesium sulphate had been added. To each of twelve of the lots of sand an addition of 5·0 grms. of finely ground hay was made, and after thorough admixture each portion was transferred to a large narrow necked bottle with a cotton wool plug. All the bottles were then heated to 95° for one hour, and after the sand had cooled down the bottles were divided into four lots and treated as follows:

I. Sand alone. Heated and then reinoculated.

II. Sand with hay dust. Heated but not inoculated.

III. Sand and hay dust. Heated and then inoculated with putrefactive organisms.

IV. Sand and hay dust. Heated and then inoculated with putrefactive organisms and Azotobacter.

Providing secondary effects did not come into play, the difference between sets I and II might be attributed to the nitrogen compounds liberated from the hay by heat: that between sets II and III would be due to the putrefactive organisms added; any advantage derived from the presence of Azotobacter would be expressed by the difference between sets III and IV.

The putrefactive organisms referred to above were obtained by transference of the colonies occurring on six nutrient agar plates which had been inoculated with a suspension of garden soil, and to this mixed culture a few drops of a sub-culture of nitrifying organisms in mineral salt solution were added. The term "putrefactive" is employed here in a very broad sense, the main object being to secure a mixed and effective bacterial flora not containing Azotobacter. One cubic centimetre of this suspension was applied to the sand of sets I, III and IV, but set IV

received in addition a similar quantity of a suspension of a pure culture of *Azotobacter chroococcum*. The bottles were stored in the laboratory at 15–20°, with occasional shaking, for six weeks, and their contents were then returned to the corresponding vegetation pots. From this time no special precautions against infection were observed excepting

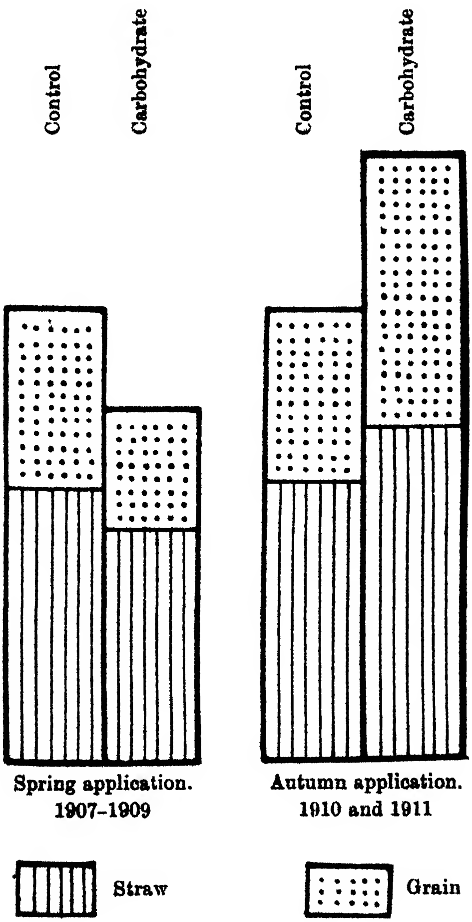

Fig. 1. Effect of carbohydrates on the yield of barley. Hoos Field experiments, 1907–1911. Relative yields.

that any seeds sown in the pots were previously treated with dilute mercuric chloride solution and only sterilised water was used for maintaining the water content of the sand.

Barley was grown as a first crop, five seeds being sown in each pot on

May 4th, 1911. Although growth in the pots of the same sets was very similar, that of the different sets varied greatly. During the first few weeks after sowing those plants in the control pots (sand alone) were distinctly superior to any of the others, there being little to choose between the rest. Towards the end of June, however, the barley in the sand inoculated with Azotobacter began to improve rapidly and at the end of three months appeared to be the best crop; this was subsequently

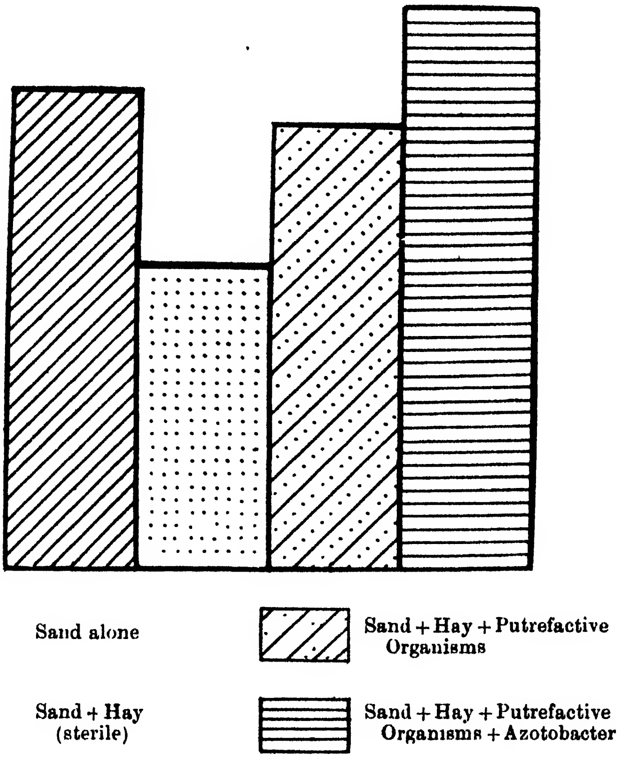

Fig. 2. Pot experiments on the utilisation of hay dust for nitrogen fixation. Production of dry matter by first crop (Barley).

confirmed by actual weighings of the dry crop. The next plants to improve were those in sand to which putrefactive organisms had been added, but those in sand (with hay) without introduced organisms remained comparatively small and weakly during the whole vegetation period.

A second crop of rye was then sown in all the pots and here again the pots with Azotobacter possessed the most vigorous vegetation; in

TABLE II. *Utilisation of Hay for Nitrogen Fixation.*

Pots and treatment	Sand: Final nitrogen, Per cent.	Sand: Final nitrogen, Total mgms.	First crop (Barley): Total dry matter (grms.)	First crop (Barley): Nitrogen in dry matter (per cent.)	First crop (Barley): Total nitrogen in dry matter (mgms.)	Second crop (Rye): Total dry matter (grms.)	Second crop (Rye): Nitrogen in dry matter (per cent.)	Second crop (Rye): Total nitrogen in dry matter (mgms.)	Total dry matter in first and second crops (grms.)	Total nitrogen in first and second crops (mgms.)	Total nitrogen in crops and sand (mgms.)
Sand alone	0·0028	400	7·34	0·6685	49·06	2·53	0·8386	21·20	9·87	70	470
Sand + hay + putrefactive organisms + Azotobacter	0·0047	671	8·56	0·8605	73·65	11·38	0·4572	54·07	19·94	127	798
Sand + hay + putrefactive organisms	0·0038	543	6·82	0·6953	47·42	7·07	0·4115	29·09	13·89	76	619
Sand + hay (sterile)	0·0037	528	4·65	0·5679	26·41	5·37	0·5483	29·44	10·02	56	584

TABLE III. *Utilisation of Sugar for Nitrogen Fixation (Sand Series).*

Pots and treatment	Sand: Final nitrogen, Per cent.	Sand: Final nitrogen, Total mgms.	First crop (Barley): Total dry matter (grms.)	First crop (Barley): Nitrogen in dry matter (per cent.)	First crop (Barley): Total nitrogen in dry matter (mgms.)	Second crop (Rye): Total dry matter (grms.)	Second crop (Rye): Nitrogen in dry matter (per cent.)	Second crop (Rye): Total nitrogen in dry matter (mgms.)	Total dry matter in first and second crops (grms.)	Total nitrogen in first and second crops (mgms.)	Total nitrogen in crops and sand (mgms.)
Sand alone	0·0028	400	7·34	0·6685	49·06	2·53	0·8386	21·20	9·87	70	470
Sand + sugar + Azotobacter	0·0056	799	7·48	0·7347	54·95	4·61	0·8424	38·83	12·09	94	893
Sand + sugar (sterile)	0·0035	500	2·01	0·5590	11·23	2·38	0·9470	22·53	4·39	34	534

contradistinction to the first crop this difference was evident from the earliest stages. The results of these experiments are briefly given in Tables II and V, and the dry matter production is diagrammatically represented in Figs. 2 and 3.

As the result of treatment with putrefactive organisms alone, no definite increase in the nitrogen of the sand and crop is evident, but where Azotobacter was additionally present the nitrogen content of the sand was distinctly increased. After deducting the amount of nitrogen supplied in the hay (148 mgrm.) there still remains a surplus of about 120 mgrm.

Similarly, the amount of nitrogen in the first and second crops was increased from 49 mgrm. to 73 mgrm. and from 21 to 56 mgrm. respectively. Taking all the gains into account one finds that by the utilisation of the hay dust as a source of energy, a nett increase of 180 mgrm. occurred. When bacteria were not added but must have been eventually introduced by air infection the crop nitrogen was reduced from 49 to 26 mgrm. in the first crop, and only rose by 8 mgrm. in the following rye crop.

On the whole one must conclude either that the nitrogen supplied in the hay dust was extremely badly utilised or that some degeneration process resulting in the withdrawal or loss of nitrogen took place during the initial period of growth. Since the pots with Azotobacter also passed through this stage of depression it is probable that a more accurate gauge of the intensity of assimilation would be obtained by a comparison between the set with Azotobacter and that without any bacteria added.

If the fact be taken into consideration that such gains occurred within six to seven months from the time of treatment, the degree of utilisation may be regarded as fairly satisfactory.

Utilisation of Sugar for Nitrogen Fixation.

In order to allow of some comparison of the value of plant residues and that of some directly available source of energy, two further sets of pot experiments were carried out in which sugar was applied to the sand at the rate of 0·2 per cent. The general preliminary treatment was the same as in the preceding sets—potassium phosphate and calcium carbonate being supplied. After being sterilised, the sand belonging to one set of pots was maintained in a sterile condition, but the other portion was inoculated with a pure culture of Azotobacter. Later treatment, sowing, harvesting, etc. was the same as with the hay dust sets of pots.

The initial depressing effect of the treatment with sugar on subsequent growth was even more pronounced than with hay dust, and by

the time the first crop was removed the plants with sugar and Azotobacter had merely succeeded in equalling those in the control pots, but the crop in sand and sugar which had been kept free from infection up to the time of sowing was only one-third of the control. The yield of

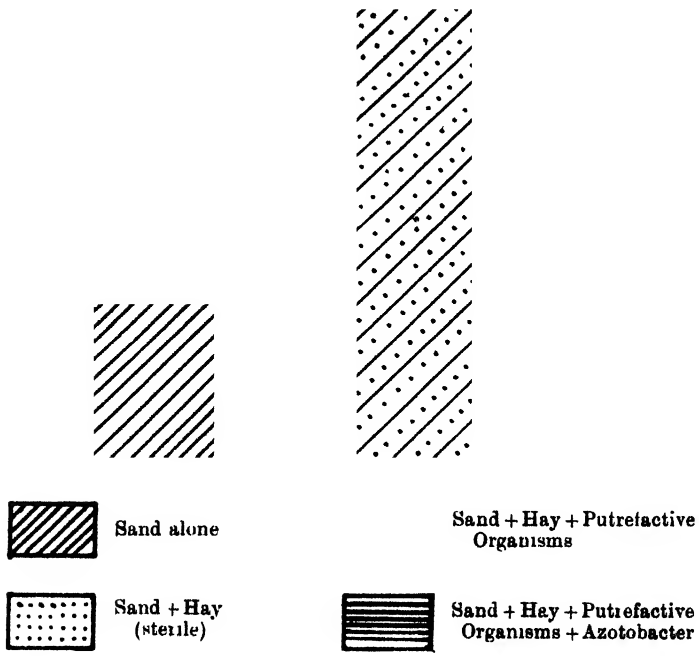

Fig. 3. Pot experiments on the utilisation of hay dust for nitrogen fixation. Production of dry matter by second crop (Rye).

rye, as a second crop, was distinctly better with Azotobacter, but by far the largest proportion of nitrogen assimilated was found to remain in the sand. It is, however, reasonable to suppose that this is due largely to the fact that putrefactive organisms were not supplied to these sugar

pots, and in their absence the breakdown of the nitrogen compounds stored up in the Azotobacter cells would therefore proceed but slowly.

As a result of inoculation with Azotobacter the nitrogen in the two crops was increased from 34 mgrm. (without inoculation) to 94 mgrm. with Azotobacter: the total nitrogen in sand and crop showed still greater differences, being 460 mgrm. in the control pots as against 534 and 893 mgrm. in the treated pots. (Table III.)

Further experiments consisted in the application of sugar to soil in pots similar to those used in the above experiments. Since Azotobacter is normally present in these soils it was not necessary to add this organism nor was it considered desirable to introduce other factors by subjecting the soil to heat prior to the application of the sugar, as was done in the case of the sand cultures. One set of pots was retained as control, and of the two sets treated with sugar, one was stored at a temperature of 5-10° C. whilst the other was stored at between 15-30° C.

The influence of temperature on nitrogen fixation has been noted in various laboratory experiments and the general result was obtained that when the temperature falls to the region of 5° fixation comes to a standstill, whilst it is most active between 18-31°. This is well borne out by these pot experiments, the plants of both the first and second crops grown in soils receiving sugar and incubated at 30° being not only heavier but also richer in nitrogen than those in the other two sets. The general results suffice to show the importance of supplying the source of energy when the soil temperature is favourable for the growth of Azotobacter and thus confirm those obtained in the field experiments discussed in the first part of the paper. (Table IV.)

TABLE IV. *Utilisation of Sugar for Nitrogen Fixation (Soil Series).*

	First crop (Barley)			Second crop (Rye)			Total dry matter in first and second crops (grms.)	Total nitrogen in first and second crops (mgms.)
Pots and treatment	Total dry matter (grms.)	Nitrogen in dry matter (per cent.)	Total nitrogen in dry matter (mgms.)	Total dry matter (grms.)	Nitrogen in dry matter (per cent.)	Total nitrogen in dry matter (mgms.)		
Soil alone	7·74	1·233	95·43	13·18	1·226	161·58	20·92	257
Soil + sugar (high temperature)	13·85	1·443	199·85	12·67	1·957	247·9	26·52	448
Soil + sugar (low temperature)	11·20	1·168	130·81	8·19	1·549	126·8	19·39	258

The rapidity with which the assimilated nitrogen becomes available for plant growth appears to differ with the medium employed. In the case of sand treated with sugar (or hay dust) little effect was apparent in the first crop but was marked in the second crop; with soil, the first crop was benefited most and the second crop was slightly below the control,

although it possessed a much higher nitrogen content. This is possibly due in the first place to the numerical superiority of Azotobacter already present in the soil and may also be connected with the effect exerted by traces of humates and of iron compounds on nitrogen fixation noted by other workers.

The gains of nitrogen per gram of substance supplied are given in the following table, from which it is seen that the returns from the use of sugar are somewhat higher than those obtained by the addition of hay dust. On the other hand, it must be recognised that the whole of the carbonaceous compounds in the latter material would probably not be completely oxidised within the course of the experiments. The actual return is consequently greater than would appear from the data given.

TABLE V.

Utilisation of Hay and Sugar for Nitrogen Fixation.

Treatment	Total nitrogen in sand and crop, mgrms.	Gain or loss of nitrogen as compared with control, mgrms.	Nitrogen assimilated per grm. of hay or sugar supplied, mgrms.
Sand alone	470	—	—
Sand + hay (sterilised)	584	114* = −34	—
Sand + hay + putrefactive organisms	619	149* = +1	—
Sand + hay + putrefactive organisms + Azotobacter	798	328* = +180	9·0
Sand + sugar (sterilised)	534	64	2·3
Sand + sugar + Azotobacter	893	423	15·1

* Less 148 mgrms. supplied in hay.

The Influence of Sugar and Plant Residues on Losses of Soil Nitrates.

During the course of the work described in the preceding pages, a number of instances occurred in which the possible operation of nitrogen fixation processes was effectively masked by the entrance of changes which were adverse to plant growth. This was evident in the earlier stages of the pot experiments but attained greater prominence in the field work when starch or sugar was applied to the plots in spring, and especially when a minimum interval elapsed between the application and the sowing of the crop. This is seen in the following summary of the yields of plot 4. O.

	Time of application				
	March 6th	Feb 19th	Feb. 4th	Jan. 17th	Sept (mean)
Relative yields (control = 100)	nil	71	77	91	137

Quantitative bacteriological analyses of these soils (30) showed that treatment with starch or sugar leads to very marked increases of the bacterial flora and that under field conditions these increases may persist for upwards of 8–9 months. Further work in the laboratory confirmed these results and also indicated that this increased growth occurs at the expense of the easily available nitrogen compounds of the soil. When equal quantities of sugar are supplied to two portions of soil, the one stored at 10° and the other at 30°, the former not only retains the power for denitrification for a longer period, but also tends to have a higher bacterial content possibly due in part to the absence of competition by nitrogen fixing organisms at this temperature. This is illustrated in the following table. The denitrification power was tested by incubating 25 grms. of the respective soils with 50 c.c. of 0·2 per cent. solution of sodium nitrate for 48 hours at 30°.

Treatment	Soil alone	Soil + 0·2 per cent sugar 10° C.	Soil + 0·2 per cent. sugar 30° C.	Soil + 1·0 per cent sugar 30° C.
Bacteria (millions per grm. soil) 3 weeks after treatment	12·5	161·6	24·8	61·5
Nitrate reduction (expressed as mgrm. nitrogen) 3 weeks after treatment ...	nil	10·3	8·2	14·6
Ammonia and nitrate (pts per million dry soil) 3 months after treatment ...	30·4	19·2	28·9	7·2

Thus, under the most favourable conditions for its destruction the presence of 0·2 per cent. sugar confers the power of denitrification on soils, stored at 30°, for more than three weeks, and only after a period of three months does the nitrate content of the soil so treated approach that of the untreated.

The addition of sugar induced similar changes in two other soils, one of which possessed a low, and the other a high, initial nitrate content. Treatment resulted in the production of high bacterial numbers and these were accompanied by a marked reduction in the amount of soil nitrates (equal to 46 parts per million in the case of soil II). Notwithstanding these pronounced changes, the accumulation of nitrates again occurred in less than eight weeks at laboratory temperature and the differences in the total ammonia and nitrate content of the untreated and treated at the end of the experiment were in each case less than the losses which were originally occasioned by the addition of sugar.

Soil I	Bacteria (millions per grm. of soil) At start	After 23 days	After 48 days	After 114 days	Nitrogen as ammonia and nitrate (pts per million of soil) At start	After 23 days	After 48 days	After 114 days
Soil alone	12·9	10·3	21·0	—	17	30	28	30
Soil + sugar	12·9	47·0	51·0	—	17	13	13	20

Soil II	Bacteria (millions per grm. of soil) At start	After 6 days	After 62 days	After 101 days	After 215 days	Nitrogen as ammonia and nitrate (pts per million of soil) At start	After 6 days	After 62 days	After 101 days	After 215 days
Soil alone	8·2	13 2	6·9	10·6	12·4	55	51	71	85	98
Soil + sugar	8·2	129·2	96·6	32·3	25·9	55	5	24	37	71

The addition of hay dust to the soil has a like effect. Two soils were tested, the first being an untreated soil, and the second, a toluened soil which had been stored in the laboratory for some months prior to treatment with hay dust. The results are summarised below:

	Bacteria (millions per grm. of dry soil) At start	After 7 days	After 74 days	Nitrogen as ammonia and nitrate (pts per million of soil) At start	After 7 days	After 74 days
Soil I: soil alone	7	4	12	27	25	27
soil + 0·5 % hay dust...	7	94	62	27	13	12
Soil II: soil alone	31	41	38	43	67	58
soil + 0·5 % hay dust	31	175	136	43	23	20

It cannot be assumed from these data, however, that the soil actually loses nitrogen to the extent represented by the disappearance of nitrates after treatment. Many organisms are known to have the capacity of assimilating nitrogen in the form of nitrate and of elaborating it into protein; even under the conditions most favourable for true denitrification (that is, the liberation of gaseous nitrogen) upwards of 25–30 per cent. of the total nitric nitrogen can frequently be recovered as bacterial protein. Nitrate disappearance does not therefore provide a true index of actual soil losses, and it is in fact reasonable to suppose that under conditions where the soil is otherwise likely to lose the greater part of its soluble nitrogen compounds by excessive leaching, such a retention of nitrogen in the organic form may be more or less of an asset.

Finally, sufficient evidence has been advanced to establish a close relation between the results obtained by the addition of hay dust and sugar to laboratory and field soils, and those obtained by Wagner (31), Maercker (32) and others when simultaneous applications of nitrate of soda and farmyard manure, straw, etc., were made to the soil. Although the conditions which prevailed in their experiments were decidedly abnormal

and the results have for this reason been widely discounted, it still appears to be fairly evident from the field experiments described above that soil nitrates may be definitely and adversely affected when appreciable quantities of sugar and starch, and therefore of straw, are applied to field soils in spring. The experiments cited by Warington (33), in which it was shown that an annual application of straw at the rate of 2000 lbs. per acre to some of the Rothamsted plots did not result in any definite decrease of crop, cannot be regarded as indicating the complete absence of any adverse changes, but rather that under certain conditions an equilibrium may obtain between destructive and constructive processes.

Summary.

The foregoing experiments give definite evidence, corroborative of the work of Koch, Remy and others, that the nitrogen content of sand or soil may be appreciably increased by the activity of Azotobacter when some suitable source of energy is supplied. For this purpose sugars such as dextrose and saccharose are suitable, but distinct gains have also been obtained by the use of plant residues. In laboratory experiments an increment of upwards of 6 mgrm. of nitrogen per gram of plant residues occurred, but in pot experiments gains of 9 mgrm. per gram of substance were obtained.

It is also shown that on the field scale, and in spite of the entrance of complicating factors, definite increases of crop (equal to 20-54 per cent.) resulted from the application of carbonaceous compounds (sugar) when the soil conditions were favourable. Since the difference between the action of sugar and plant residues is largely one of degree and not of type, it is reasonable to suppose that such substances as stubble, leaves, and other complex organic materials may also serve to contribute indirectly to the reserves of soil nitrogen.

The general soil conditions making for the successful operation of nitrogen fixation processes are, in addition to the supply of some source of energy, a suitable temperature, the presence of phosphates and a supply of basic material such as calcium carbonate. Even under the most favourable circumstances for nitrogen fixation, there occurs a period during which adverse processes come into play, and it is not advisable that a crop be introduced before these have run to completion.

Under unfavourable conditions and particularly during periods of low temperature, these adverse changes may persist without any subsequent entrance of soil gains.

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(*Received February* 15*th,* 1918.)

VOLUME IX APRIL, 1919 PART II

DECOMPOSITION OF CYANAMIDE AND DICYANODIAMIDE IN THE SOIL

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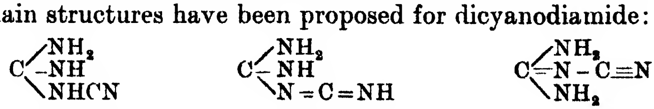
THE present investigation was undertaken to study the decomposition of cyanamide and dicyanodiamide in the soil and their specific action on plant growth. Their close chemical relationship necessitated a comparison of their respective behaviour in the soil. Dicyanodiamide is a polymer of cyanamide to which the formula $C_2H_4N_4$ is assigned. Morrell and Burgen (1) and Werner (2) have shown that the polymerisation is caused by acids or alkalis, even in small amount, but a weak alkali is substantially more effective than a correspondingly weak acid; the rate of the change is greatly increased by raising the temperature. The process does not appear to be reversible.

According to Werner cyanamide can be represented by either of the formulae (1) $CNNH_2$, (2) $NH = C - NH$. According as the equilibrium assumed to exist between these tautomeric forms in a neutral solution is displaced by an acid or base, so the polymerisation may give rise to isomeric forms of dicyanodiamide, thus:

Hofmann's formula (3) Baumann's formula (1)

In contrast to the closed-chain formulae, however, the following open-chain structures have been proposed for dicyanodiamide:



by Bamberger (5) Rathke (6) Pohl (7)

The decomposability of dicyanodiamide in the soil may throw some light upon its chemical structure; if it showed little tendency to form ammonia under these conditions, it might be more probably an imino than an amino grouping[1].

[1] Miyako (8) has shown that fatty amino compounds are ammonified much more easily than aromatic compounds, while aromatic imino compounds are much more difficultly ammonified than aromatic amino compounds.

Our investigation has shown that the two compounds behave very differently in the soil. Cyanamide readily breaks down yielding ammonia which then nitrifies in the usual way. The evidence indicates that the conversion of cyanamide nitrogen into nitrate is practically quantitative, irrespective of the nature of the soil. Cyanamide therefore serves as a valuable fertiliser. Parallel tests made in the laboratory and in the pot-culture house showed that the amount of nitrate accumulating in the soil to which cyanamide is added is identical with the amount of nitrogen taken up by the plants growing in the soil containing an equal amount of cyanamide and under generally similar conditions.

No evidence was obtained to support the views held by Immendorff (9) and Kappen (10) as to the production of dicyanodiamide from cyanamide in poor soils and those of an acid nature of low bacterial activities.

On the other hand, our experiments have demonstrated that dicyanodiamide not only fails to act as a nutrient but is actually toxic to plants. Small quantities (18 mgs. N per kilo soil) in pot-culture do not appreciably injure the higher plants, but still smaller quantities exerted no stimulating influence on growth; this affords an additional corroboration of Brenchley's (11) contention that poisonous substances do not necessarily act as stimulants when supplied in sufficiently small doses.

When dicyanodiamide is mixed with cyanamide it greatly reduces the amount of nitrate produced from the cyanamide. Careful investigation, however, brought out the striking fact that the plant succeeded in obtaining more nitrogen from the mixture than corresponded with the nitrate produced, thus:

	% added N removed in crop	% added N nitrified
Cyanamide 3 parts Dicyanodiamide 1 part	56·7	22
Cyanamide 1 part Dicyanodiamide 3 parts	23·7	5·3

Further investigations showed that the dicyanodiamide had not prevented the formation of ammonia from cyanamide; indeed it had hardly affected this reaction, but it had almost entirely inhibited the subsequent transformation of ammonia into nitrate.

The dicyanodiamide was found to be toxic to the sensitive nitrifying organisms, so that it stops the decomposition of cyanamide at the ammonia stage and inhibits the formation of nitrates.

Thus the addition of dicyanodiamide to cyanamide results in an accumulation of ammonia in the soil which does not undergo the normal oxidation to nitrates but persists as ammonia.

In like manner the addition of dicyanodiamide to soil also containing ammonium sulphate stops the oxidation normally brought out by the nitrifying organisms.

It does not appear, however, that dicyanodiamide has so drastic an effect on the other organisms of the soil, especially on those concerned in the decomposition of protein. It hardly affects the numbers developing on gelatine plates or the rate and extent of the decomposition of dried blood.

The Production of Ammonia and Nitrate from Cyanamide and Dicyanodiamide.

The cumulative evidence from the Nitrification Tests (plotted in Fig. 1) clearly indicates that the change of cyanamide nitrogen into nitrate is almost quantitative and closely approximate in this respect to ammonium sulphate. The nitrification of cyanamide, however, is appreciably slower than that of the latter compound, especially in the initial stages. This would be accounted for by the time required for the primary decomposition of cyanamide into ammonia.

Dicyanodiamide, on the other hand, has given practically no evidence of nitrification even after three months.

Cyanamide and ammonium sulphate show a general similarity. The former lags distinctly for the first 15 days, but it then gains substantially and after 35 days runs appreciably close and nearly parallel to the other.

The mixtures of cyanamide and dicyanodiamide show less nitrification than was expected from the cyanamide present, while the dicyanodiamide gave no indication of being nitrified at all. This result was obtained both with the heavy Rothamsted and the light Woburn soil (see Table V).

Crop Results.

In the vegetation experiments cyanamide produced a consistently beneficial effect on plant growth and gave results substantially in accordance with the extent of its nitrification as shown by the laboratory tests. In the Rothamsted soil it exerted no adverse action on germination in any of the quantities used, but a dressing of 100 mgs. N per kilo soil caused some retardation in the Woburn soil. It was somewhat slower in action than ammonium sulphate, especially when a small amount of dicyanodiamide was present. Its nutrient effect, however, proved approximately equal. This is also in agreement with the

average results of the field trials in Great Britain, which show that, when ammonium sulphate is expressed as 100, nitrolim is equal to 94·4. These results include some cases in which nitrolim delayed germination, or caused a temporary check to the crop, where applied wrongly as a top dressing.

The pot experiments, on the other hand, demonstrated conclusively the harmful effect of dicyanodiamide. Even in the maximum dressing, however, it did not affect germination. Its toxicity was first revealed a few days after germination by the white tips or margins of the cotyledons. The other leaves became similarly affected in turn. With small amounts the injury was confined to the extreme tips of the leaves and the plants showed more or less normal growth. The larger dressings, however, caused a progressive withering of the leaves from the tips downwards and subsequently depressed the growth below that of the controls, or finally, in sufficient quantity, killed the plants. The toxic effect was slightly more pronounced in the light Woburn than in the heavy Rothamsted soil.

In combination with cyanamide the greater the proportion of dicyanodiamide nitrogen, the lower the yield. Where a mixture of cyanamide and dicyanodiamide was used in the proportion of one part cyanamide and three parts dicyanodiamide, the depressing effect of the dicyanodiamide more than counterbalanced the beneficial effect of the cyanamide.

Similar results were obtained in the field trials (p. 133).

The pot experimental results with mustard and barley were:

	Mustard. Rothamsted soil 50 mgs. N per kilo soil applied		Barley. Woburn soil 25 mgs. N per kilo soil applied	
	Relative dry weights	% added N removed in crop	Relative dry weights	% added N removed in crop
Control	100	—	100	—
Ammonium sulphate ...	322	87·8	119	60·6
1. Cyanamide alone ...	276	73·0	112	56·4
2. Cyanamide 3 parts, Dicyanodiamide 1 part	242	62·9	95	32·8
3. Cyanamide 1 part, Dicyanodiamide 3 parts	113	23·7	102	25·2
4. Dicyanodiamide	65	9·7	—	—

It is interesting to note the high percentages of nitrogen recovered in the mustard crop from the ammonium sulphate and pure cyanamide dressings, considering that the roots were not included in the analysis.

When cyanamide alone is used there is a very close relationship between the amount of nitrogen converted into nitrate in the laboratory tests and the amount of nitrogen assimilated by the plant in the pot-culture tests. But when dicyanodiamide is present this close relationship no longer holds. Assuming that the nitrification of the soil organic matter is not materially affected by the cyanamide, the following quantities of nitrogen were assimilated in the mustard pot experiments and nitrified in the laboratory tests:

100 mgs. N per kilo soil applied	Pot experiments % added N assimilated	Nitrification Tests. % added N nitrified after 84 days
1. Cyanamide alone ...	64·3	65·1
2. Cyanamide 3 parts, Dicyanodiamide 1 part	56·7	22·0
3. Cyanamide 1 part, Dicyanodiamide 3 parts	23·9	5·3
4. Dicyanodiamide alone ...	0	5·6

From the mixtures of cyanamide and dicyanodiamide the plants have taken up substantially more nitrogen than there was nitrate produced. The comparatively small amount of nitrification which has occurred in these cases suggested some adverse effect of the dicyanodiamide on the nitrification of the cyanamide.

The retardation of the nitrification of cyanamide in the presence of dicyanodiamide was then investigated using cyanamide alone, 50 mgs. N per kilo soil, and an equal quantity of cyanamide in addition to a small amount (15 mgs. N per kilo soil) of dicyanodiamide. The results show a marked depressing effect of the dicyanodiamide on the nitrification of the cyanamide.

	N as Nitrate present per million dry soil					N as NH_3 present per million dry soil		
	At start	After 6 days	After 18 days	After 42 days	After 162 days	After 18 days	After 42 days	After 162 days
Control (no N) ...	15·0	21·6	21·5	25·6	31·6	4	5	1
Cyanamide alone	—	25·9	60·2	74·2	81·6	3	5	1·5
Cyanamide + dicyanodiamide	—	20·0	19·9	28·4	56·3	23·5	26	16·8

Added 50 mgs. cyanamide N per kilo soil = 60 parts of N per million dry soil
15 mgs. dicyanodiamide N per kilo soil.

Nitrification of the cyanamide has been almost entirely inhibited by the dicyanodiamide for 42 days, after which it proceeds only at a slow rate and is not complete after 162 days. There is, however, a notable production of ammonia from cyanamide in the presence of dicyanodi-

amide. This indicates that the decomposition begins normally; ammonia is formed but the sensitive nitrifying organisms are inhibited by the dicyanodiamide.

To ascertain whether this hypothesis be correct, the effect of dicyanodiamide on the nitrification of ammonium sulphate was next determined. The results (plotted in Fig. 3) show a marked retardation of the nitrification of ammonium sulphate in the presence of dicyanodiamide.

	N present as Nitrate per million dry soil					N present as NH_3 per million dry soil				
	At start	After 7 days	After 21 days	After 42 days	After 94 days	At start	After 7 days	After 21 days	After 42 days	After 94 days
Control	8·8	12·4	18·8	17·5	20·6	2·6	3·8	1·3	3·9	5·2
Ammonium sulphate alone	—	19·5	33·7	41·4	55·0	—	28·5	16·8	16·5	7·9
Ammonium sulphate + dicyanodiamide	—	10·9	13·5	13·2	14·9	—	35·0	32·4	34·1	30·6

Added 50 mgs. ammonium sulphate N per kilo soil − 60 parts of N per million dry soil. 15 mgs. dicyanodiamide N per kilo soil.

For the first 42 days the mixture of ammonium sulphate and dicyanodiamide shows but a small production of nitrate and practically no reduction in ammonia content. Even after 94 days the action has only proceeded to an insignificant extent.

We are now able to explain the discrepancy between the pot and the laboratory nitrification results in the case of the mixture of cyanamide and dicyanodiamide (Figs. 1 and 2). In the laboratory tests the cyanamide breaks down to ammonia, but the action goes no further because the nitrifying organisms are adversely affected by dicyanodiamide; therefore nitrates do not accumulate. In the pots the plants are able to make use of the ammonia formed, either because the retardation of nitrification does not operate to the same extent as in the laboratory tests or the plants have taken up some of their nitrogen in the form of ammonia. This inhibition of nitrification by dicyanodiamide no doubt explains the slower action of cyanamide on plant growth in the presence of even a small amount of this compound.

Influence of Dicyanodiamide on the Soil Bacteria.

Experiments were next made to ascertain whether dicyanodiamide affects other organisms of the soil besides the nitrifying group.

Conjointly with the nitrification tests counts were made of the

number of bacteria capable of development on gelatine plates. None of the dressings, however, seems to have exerted any marked effect on these numbers. Some stimulation of the bacteria in the initial period may have been caused by the cyanamide in series 1 and 3, but no marked depression of the numbers was produced by dicyanodiamide. In series 1, where the largest dressing was used, the numbers with the pure dicyanodiamide show practically no greater variation than the natural fluctuation of the control:

Bacteria. Millions per gram of fresh soil

	At start	After 5 days	After 15 days	After 35 days
Control	9·1	16·5	14·0	10·9
Soil treated with dicyanodiamide (100 mgs. N per kilo soil)	—	17·8	14·0	8·7

Similar results were obtained in the other series.

Effect of Dicyanodiamide on the Ammonification of Dried Blood.

The influence of dicyanodiamide on the ammonifying organisms was studied by determining the rate of ammonification of dried blood. The results were:

	N present as Nitrate per million dry soil					N present as NH_3 per million dry soil				
	At start	After 7 days	After 21 days	After 42 days	After 94 days	At start	After 7 days	After 21 days	After 42 days	After 94 days
Control	8·8	12·4	18·8	17·5	20·6	2·6	3·8	1·3	3·9	5·2
Dried blood ...	—	15·7	25·5	28·8	36·1	—	5·8	4·5	9·7	3·8
Dried blood + dicyanodiamide	—	10·4	12·5	14·7	13·3	—	9·1	17·4	20·5	18·2
Dicyanodiamide alone	—	9·1	8·5	13·3	11·7	—	3·9	3·2	5·2	3·9

Added 50 mgs. dried blood N per kilo soil = 60 parts of N per million dry soil.
15 mgs. dicyanodiamide N per kilo soil.

	Total N as Nitrate + NH_3 per million dry soil					Bacteria. Millions per gram of fresh soil				
	At start	After 7 days	After 21 days	After 42 days	After 94 days	At start	After 7 days	After 21 days	After 42 days	After 94 days
Control	11·4	16·2	20·1	21·4	25·8	12·0	11·0	14·1	8·3	20·0
Dried blood ...	—	21·5	30·0	38·5	39·9	—	15·7	19·4	24·6	23·0
Dried blood + dicyanodiamide	—	19·5	29·5	35·2	31·5	—	10·4	15·2	24·0	19·2
Dicyanodiamide alone	—	13·0	11·7	18·5	15·6	—	11·0	8·3	13·7	14·0

The above results (plotted in Fig. 5) do not indicate that the ammonification of the dried blood has been appreciably retarded by the dicyanodiamide. It is noteworthy, however, that while the dicyanodiamide alone has produced no depression of the bacterial numbers, it has caused a temporary check to the multiplication of the organisms by the dried blood. It would therefore appear that dicyanodiamide has an adverse action on some of the ammonifying bacteria in the soil; this is also suggested by the slight initial depression of the decomposition of dried blood in the presence of dicyanodiamide.

The readiness with which cyanamide is converted into nitrate in contrast to the marked resistance shown to this change by dicyanodiamide demonstrates conclusively that the latter is not normally produced from cyanamide in the soil. Further, our results afford no support to the hypothesis of Immendorff and Kappen as to the occurrence of polymerisation in poor soils. Reference to Table V will show that the production of nitrate from cyanamide has proceeded approximately to the same extent in the poor Woburn soil as in the Rothamsted soil.

The experiments, on the other hand, provide no indication of any appreciable formation of ammonia from dicyanodiamide in the soil. This fact suggests the possibility of a di-imino formula for dicyanodiamide.

EXPERIMENTAL.

Mustard Pot Experiments.

These experiments were carried out concurrently in two series, using Rothamsted soil (a heavy loam) from Great Knott Field, after being passed through a ½ inch sieve and thoroughly mixed with 10 per cent. sand. The soil contained ·1 per cent. calcium carbonate. The nitrogenous dressings[1] taken for comparison were:

I. Cyanamide.
II. Cyanamide 3 parts,
Dicyanodiamide 1 part.
III. Cyanamide 1 part,
Dicyanodiamide 3 parts.
IV. Dicyanodiamide.

[1] The pure cyanamide was applied in the form of fresh nitrolim, in which the calcium cyanamide had undergone practically no change.

The mixtures of cyanamide and dicyanodiamide were applied respectively in samples of old nitrolim, in which the cyanamide had become transformed into dicyanodiamide to the extent shown by the mixtures in question.

The dicyanodiamide was prepared in an approximately pure state from a sample of nitrolim and found to contain over 66 per cent. nitrogen as compared with the theoretical 66·6 per cent.

In series I the above dressings were tested respectively at the rates of 50 and 100 mgs. N per kilo soil and in series II the last three were compared at 200 and 100 mgs. N per kilo soil. In each series the pots were divided into sets of three, and all received 1 gram dicalcium phosphate and ·5 gram potassium sulphate per kilo soil. Controls without nitrogen were included in each series.

The fertilisers were applied to the soil and the pots filled on 6th September. The seed was sown the following day, 10 seeds in the small and 20 in the large pots. The seedlings were subsequently thinned down to 7 in the small pots and 14 in the large. The pots received measured equal quantities of water. The crop was harvested on the 20th December and the yields of dry matter etc. are set out under Table I.

Barley Experiments.

Each pot held 20 kilos soil, and the nitrogenous dressings were as used in the mustard series, viz. 25 mgs. N per kilo soil both on the heavy Rothamsted soil and on the light Woburn soil. The latter contained practically no calcium carbonate and was slightly acid in reaction. In all cases 1 gram bone ash, and ½ gram potassium sulphate were added per kilo of soil. The complete dressings were applied on 28th April and the seed sown on 30th April. Five plants were grown in each pot. The crop was harvested on the 18th July and the results are placed under Table II.

Rye Experiments.

This series was undertaken to ascertain whether dicyanodiamide exerted a stimulating action on plant growth, when supplied in a sufficiently small amount to be no longer deleterious. Dicyanodiamide was applied at the rate of 12½ mgs. N per kilo soil: it was tested alone and also in conjunction with cyanamide at the rate of 25, 50 and 100 mgs. N per kilo soil. The Rothamsted and Woburn soils were used.

All the pots received 2 grams superphosphate and ½ gram potassium sulphate per kilo of soil. The dressings were applied on the 30th October and the seed sown on the 1st November. In one case the dicyanodiamide alone was also applied in the aqueous solution as a top-dressing in the early stages of growth. The crop was harvested in both sets on the 24th May, and the yields of dry matter etc. are placed under Table III.

Barley-Field Trials.

The following nitrogenous dressings were tested on a heavy loam on the Rothamsted Farm at the rate of 30 lbs. nitrogen per acre:

(1) Ammonium sulphate.
(2) Cyanamide.
(3) Cyanamide 9 parts,
Dicyanodiamide 1 part.
(4) Cyanamide 1 part,
Dicyanodiamide 9 parts.

The soil contained 1·7 per cent. carbonate of lime. The plots, measuring $\frac{1}{10}$ acre, were arranged in duplicate, including controls without nitrogen, and all received 3 cwts. superphosphate per acre. Both dressings were applied to the plots on 26th March, that is, 10 days before sowing, in order to avoid any possible retardation of germination by the cyanamide. The seed was sown on 5th April and the crop harvested on 6th September.

The yields are set out under Table VIII.

The above experiments showed that in pots dicyanodiamide caused no appreciable injury to the plants when supplied in quantities not exceeding 18 mgs. N per kilo soil. In larger amounts, however, it proved increasingly harmful and depressed the yields more or less proportionately.

In the actual field no significant injurious effect was produced by a dressing of dicyanodiamide, equivalent to 27 lbs. nitrogen per acre.

The influence of dicyanodiamide on plant growth has previously been investigated by several experimenters, and a brief résumé of the results may be given here. M. Gerlach and P. Wagner (12) and Immendorff (13) were the first to observe its harmful effect on plants, but have given no details as to the amounts used and the quantitative effect on the yield.

In Seelhorst and Müther's (14) experiments the effect of dicyanodiamide varied with the soil: on a sandy soil barley showed white tips and died several days after germination. In a loam the plants developed nearly normally although retaining the white tips.

R. Perotti (15) found that dressings of dicyanodiamide not exceeding 1 gram in 1400 grams soil produced considerable increases in the yield of wheat, buckwheat and flax, and concluded that the value of nitrolim depended upon the formation of dicyanodiamide in the soil. C. Ulpiani (16) took a similar view.

O. Loew (17) observed a gradual withering of the leaves from the tips downwards in young barley plants grown in a nutrient solution containing 0·5 per cent. dicyanodiamide. In a 0·2 per cent. solution, on the other hand, *Elodea* was not only not injured but it assimilated and used the dicyanodiamide as a source of nitrogen. In a later investigation Loew concluded that (1) in a sterilised soil dicyanodiamide and ammonium sulphate were equally effective in grain formation, (2) in an unsterilised soil, on the other hand, barley makes little growth: this difference was attributed to injurious substances formed from the dicyanodiamide by bacteria, (3) the withering of the tips of the leaves was attributed to an accumulation there of dicyanodiamide.

In water-culture K. Aso (18) found dicyanodiamide at the concentration of ·01 per cent. served as a source of nitrogen for plants. In soil, however, it was toxic at the rate of 5 grams in 10 kilos soil but had good effects in smaller quantities.

Inouje (19) obtained similar results: dicyanodiamide at the rate of 1 gram N per 8 kilos soil was injurious especially to the young plants, while at one-third this rate it was beneficial.

A. Stutzer and F. Reis (20) found that dicyanodiamide had a marked injurious effect on the germination of both oats and barley. In pot experiments using quartz sand, dicyanodiamide at the rate of 30–45 kilos per hectare proved highly deleterious to various plants. Maize grown in loam gave only one-tenth of the yield with dicyanodiamide as with nitrate.

In pot experiments by Ch. Brioux (21) dicyanodiamide at the rate of 50 kilos N per hectare depressed the yield of buckwheat 50 per cent. below that of the controls without nitrogen in one case and 37 per cent. in another. Like Immendorff he noted the withering of the tips and margins of the leaves of the treated plants.

E. Truninger (22) found that the injurious influence of dicyanodiamide at an early stage could be largely overcome by the simultaneous application of a readily assimilable nitrogen compound. He was disposed to attribute the unfavourable influence of dicyanodiamide to its great stability in the soil, as no nitrification was evident after two months.

Pfeiffer and Simmermacher (23) have shown in a recent paper that a gradual rise in the proportion of dicyanodiamide nitrogen in the nitrogenous dressing above ·16 gram in 15 kilos soil resulted in increasing amounts of injury to the plants and corresponding reduction in the yield of oats grown in pots. The germination, however, was not

affected even by the highest amount employed, viz. 1·5 grams per 15 kilos soil. The toxic effect was less marked in a loam than in a mixture of sand and loam. Only a small proportion of the dicyanodiamide nitrogen was utilised in the plant and the heavier dressings caused only a "useless storing up" of proteins in the leaves and stems to which the injury to the plants was attributed.

J. A. Voelcker (24) found that dicyanodiamide at rates from ·23 to ·69 cwt. per acre had an increasing depressing effect on mustard grown in pots. Dicyanodiamide injured mustard much more than barley and barley more so than wheat; the effect of dicyanodiamide was more or less similar whether applied at sowing or as a top dressing.

The Production of Nitrate from Cyanamide and Dicyanodiamide.

For the nitrification tests the same soil was employed as in the pot experiments. The bulk was first thoroughly mixed together and the moisture content raised, if necessary, to the optimum level for nitrification, viz. about 15 per cent. The soil was next passed through a 3 mm. sieve, weighed out into lots of 800 grams and directly transferred to wide-mouthed bottles of 40 ozs. capacity. The bottles were then divided into sets of four, and while one set was kept as controls without nitrogen, the others received equal amounts of nitrogen in the various forms to be tested. After applying the nitrogen to the soil the bottles were plugged with sterile cotton wool and afterwards kept in a dark cellar at the ordinary laboratory temperature. At the beginning a determination of the nitrates in the untreated soil was made, and at each of the periods in question the nitrates were determined in one bottle from each set.

Where necessary, soils were taken also from the same bottles for the determination of ammonia and bacterial counts.

The Determination of Nitrates in the Soil.

In the first series of nitrification tests the nitrates were determined by the reduction method (25). The soil was extracted in the usual way with distilled water and the solution concentrated by boiling to a small volume with a trace of added magnesia. The nitrates were reduced in the concentrated soil solution by the zinc-copper couple and the ammonia afterwards determined in the usual way. Soil treated with dicyanodiamide was found to give by this method at the end of the different periods a practically constant slight excess of ammonia over

that of the controls. This indicated that the ammonia was originating from a slight decomposition of the compound itself as the direct effect of the reagents, and it was accordingly decided to test the accuracy of the method in presence of the nitrogenous dressings used. Equivalent amounts of nitrogen were, therefore, added to the soil in the same proportions and determination of the nitrates made forthwith before any nitrification of the added materials could take place. The results showed the method was subject to the following errors with the different dressings:

I.	Cyanamide	12·7 %
II.	Cyanamide 3 parts Dicyanodiamide 1 part	14·1 %
III.	Cyanamide 1 part Dicyanodiamide 3 parts	4·9 %
IV.	Dicyanodiamide ..	7·1 %
V.	Ammonium sulphate ...	1·4 %

The above table should therefore be considered in association with the nitrification results under Table IV and plotted in Fig. 1. It must be noted, however, that with the rapid conversion of cyanamide to ammonia, even in the presence of dicyanodiamide, the above errors would be substantially reduced even at the first period with the first and second dressings and would probably only continue to hold good in respect to the proportion of dicyanodiamide present. For further corroboration, however, the nitrates were also determined at the final period by the phenol-sulphonic acid method, and the figures, also included in the table, confirm substantially the results obtained by the reduction method.

In the subsequent tests the phenol-sulphonic acid method was adhered to throughout.

Percentage Nitrification of the added Nitrogen.

To arrive at the percentages nitrified of the added nitrogen a difficulty was introduced through the rise in the nitrate content of the control during the progress of the experiment and the consequent inability to determine what figure should be deducted from the amounts of nitrate in the treated soils. As a comparison of the results will show, the control starting with a relatively low nitrate content has shown in most cases a steady rise in nitrates, amounting to 10–15 parts N per million dry soil, according to the length of time the series was allowed to run. In such a case it would be unjustifiable to assume that the nitrification

of the organic matter in the soil would proceed to the same extent in the treated as in the control soils: the amount of soil organic matter nitrified in the treated soils might conceivably depend on the relative availability of the added nitrogen. In cases such as series I and II in the Rothamsted soil, where the control has remained more or less constant throughout, the matter is simplified and the percentages might then be approximately determined by deduction of the highest control as has been done in the series in question. In other cases a general comparison only can be made between the amounts of nitrates formed in the control and the experimental sets.

The Determination of Ammonia in the Soil.

The amounts of ammonia in the soil were determined by Folin's aeration method, which has been modified and adapted for soil investigation by Potter and Snyder (26). It was essential to success that the method should entail no formation of ammonia from the cyanamide or dicyanodiamide. Neither of these substances, however, gave evidence of any appreciable hydrolysis under the conditions in question. Further, the amount of ammonia found by this method in the Rothamsted soil satisfactorily agreed with that recovered by extraction with dilute hydrochloric acid. The results were:

60 parts N per million dry soil added

	N recovered per million dry soil	
	Rothamsted	Woburn
Soil alone (aeration)	2·0	1·1
„ (extraction with dilute HCl)	1·6	—
Soil and cyanamide	4·1	1·9
Soil and dicyanodiamide	1·9	1·1
Soil and mixture of cyanamide and dicyanodiamide	1·9	1·9
Soil and ammonium sulphate	37·5	42·9

The above results show that the cyanamide and dicyanodiamide compounds remain comparatively stable under the conditions of the experiment. A slight tendency to decomposition is evident only with the cyanamide dressing in the Rothamsted soil. The figures for ammonium sulphate reveal the difficulty in recovering the whole of the ammonia by reason of its absorption and retention by the soil. By adopting, however, the same definite period for aeration duplicate samples were found to give satisfactorily concordant results and consistently show an approximate recovery of 60 per cent. of the added ammonia in the Rothamsted soil.

Summary.

Cyanamide readily breaks down in the soil yielding ammonia, which then nitrifies in the usual way. The conversion of cyanamide nitrogen into nitrate is practically quantitative, and its effectiveness as a fertiliser is approximately equal to that of ammonium sulphate.

Dicyanodiamide has given no evidence of nitrification in the soil even after several months. On the contrary, it is actually toxic to plants, although in small amounts it causes no appreciable injury. It does not affect germination at any of the concentrations used.

Dicyanodiamide is also toxic to the nitrifying organisms and stops the normal oxidation of ammonia in soils containing ammonium sulphate. It likewise inhibits the transformation into nitrate of the ammonia produced from cyanamide in the soil and causes an accumulation of ammonia under these conditions. It does not sensibly retard the formation of ammonia from cyanamide.

Dicyanodiamide does not appear to affect so drastically the other organisms of the soil, especially those concerned in the decomposition of protein. It exerts little influence upon the numbers developing on gelatine plates or the rate and extent of the decomposition of dried blood.

In conclusion the writer desires to acknowledge his indebtedness to Dr E. J. Russell, who kindly suggested the lines of the investigation, for his helpful advice throughout its progress, to Dr W. E. Brenchley, for much valuable assistance in connection with the pot-culture experiments, and also to Mr B. F. Davis, F.I.C., who kindly supplied samples of the various products and made many useful suggestions in the course of the work.

TABLE I. *Dry Matter and Amounts of Applied Nitrogen Recovered in Mustard Crop.*

Treatment	Dry matter. Grams per pot (mean of 3 pots)	% N in dry matter	Nitrogen removed. Grams per pot	% applied nitrogen removed in crop
Series I:				
Large pots (20 kilos soil), N added = 50 mgs. per kilo soil or 1 gram per pot				
Control (no N)	16·70	1·94	·323	—
Ammonium sulphate	51·7	2·32	1·201	87·8
1. Cyanamide	46·0	2·29	1·053	73·0
2. Cyanamide 3 parts, Dicyanodiamide 1 part ...	40·5	2·35	·952	62·9
3. Cyanamide 1 part, Dicyanodiamide 3 parts ...	18·8	2·98	·560	23·7
4. Dicyanodiamide alone ...	10·8	3·87	·420	9·7
N added = 100 mgs. per kilo soil or 2 grams per pot				
1. Cyanamide	53·15	3·03	1·610	64·3
2. Cyanamide 3 parts, Dicyanodiamide 1 part ...	40·70	3·58	1·457	56·7
3. Cyanamide 1 part, Dicyanodiamide 3 parts ...	13·50	5·93	·801	23·9
4. Dicyanodiamide alone ...	3·80	5·41	·206	0
Series II:				
Small pots (10 kilos soil), N added = 100 mgs. per kilo soil or 1 gram per pot				
Control (no N)	9·50	1·73	1·640	—
2. Cyanamide 3 parts, Dicyanodiamide 1 part ...	17·05	3·95	·673	50·9
3. Cyanamide 1 part, Dicyanodiamide 3 parts ...	7·20	6·34	·456	29·2
4. Dicyanodiamide alone ...	3·40	7·76	·264	10·0
N added = 200 mgs. per kilo soil or 2 grams per pot				
2. Cyanamide 3 parts, Dicyanodiamide 1 part ...	12·25	6·64	·813	32·5
3. Cyanamide 1 part, Dicyanodiamide 3 parts ...	plants killed	—	—	—
4. Dicyanodiamide alone ...	,,	—	—	—

TABLE II. *Dry Matter and Amounts of Applied Nitrogen Recovered in Barley Crop.*

N added = 25 mgs. per kilo soil or ·5 gram per pot.

Treatment	Dry matter. Grams per pot (mean of 2 pots)	% N in dry matter	Nitrogen removed Grams per pot	% applied nitrogen removed in crop
Rothamsted Soil.				
Control	36·05	1·61	·580	—
1. Cyanamide	33·28	2·06	·686	21·2
2. Cyanamide 3 parts, Dicyanodiamide 1 part ...	34 72	1·92	·667	17·4
3. Cyanamide 1 part, Dicyanodiamide 3 parts ...	34·22	1·86	·636	11·2
Woburn Soil.				
Control	20·59	1·71	·352	—
Ammonium sulphate	24·92	2 63	·655	60·6
1. Cyanamide	23·24	2·73	·634	56·4
2. Cyanamide 3 parts, Dicyanodiamide 1 part ...	19 83	2·60	·516	32·8
3. Cyanamide 1 part, Dicyanodiamide 3 parts ...	21·16	2·26	·478	25·2

TABLE III. *Effect of small amount of Dicyanodiamide on Growth of Rye.*

Treatment per pot	Rothamsted soil: Dry matter. Grams per pot. (Mean of 3 pots)	Rothamsted soil: Relative dry wts. Control =100	Rothamsted soil: % N in dry matter	Rothamsted soil: N recovered in crop. Grams	Woburn soil: Dry matter. Grams per pot. (Mean of 3 pots)	Woburn soil: Relative dry wts. Control =100	Woburn soil: % N in dry matter	Woburn soil: N recovered in crop. Grams
Control	28·9	100	·67	·193	29·8	100	·64	·191
Dicyanodiamide alone (·125 gram N)	28·0	97	·72	·201	30·2	101	·66	·199
Dicyanodiamide alone (·125 gram N) (applied in aqueous solution as top-dressing)	28·2	98	·75	·211	—	—	—	—
Cyanamide alone (·25 gram N)...	34·7	120	·91	·316	38·4	129	·90	·346
Cyanamide (·25 gram N) + Dicyanodiamide (·125 gram N)	36·6	126	·83	·304	35·0	118	·98	·343
Cyanamide alone (·5 gram N)	37·9	131	1·19	·451	35·5	119	1·35	·479
Cyanamide (·5 gram N) + Dicyanodiamide (·125 gram N)	37·3	130	1·18	·440	38·0	128	1·29	·490
Cyanamide alone (1 gram N) ...	38·1	132	1·81	·689	34·6	116	1·90	·657
Cyanamide (1 gram N) + Dicyanodiamide (·125 gram N)	36·9	128	1·83	·675	30·9	104	2·09	·646

TABLE IV. *Results of Nitrification Tests and Bacterial Counts. Rothamsted Soil.*

N added = 100 mgs. per kilo soil or 117 parts per million dry soil.

Treatment	N as nitrate present per million dry soil. Control (at start) 22·3: After 5 days	After 15 days	After 35 days	After 84 days: Reduction	After 84 days: Colorimetric	Percentage of added N nitrified: After 5 days	After 15 days	After 35 days	After 84 days: Reduction	After 84 days: Colorimetric	Bacteria. Millions per gram of fresh soil. Control (at start) 9·1: After 5 days	After 15 days	After 35 days
Control...	22·3	21·5	21·0	24·3	18·0	—	—	—	—	—	16·5	14	10·9
Ammonium sulphate...	55·0	76·0	92·0	103·4	116·1	28·0	45·8	59·5	69·3	83·8	13·5	14·4	13·8
1. Cyanamide... ...	33·9	57·5	84·1	98·5	103·3	9·0	30·0	52·8	65·1	72·9	20·7	9·0	10·0
2. Cyanamide 3 parts Dicyanodiamide 1 part	31·5	35·5	39·9	48·1	49·1	7·9	11·3	15·0	22·0	26·6	21·2	9·0	10·0
3. Cyanamide 1 part Dicyanodiamide 3 parts	29·8	31·0	30·3	28·5	20·7	6·4	7·4	6·8	5·3	2·3	20·5	9·6	8·9
4. Dicyanodiamide ...	30·9	28·4	30·6	28·9	16·2	7·3	5·2	7·0	5·6	0	17·8	14·0	8·7

TABLE V. *Showing the Results of Nitrification Tests in Rothamsted and Woburn Soils.*

N added = 25 mgs. per kilo soil = 29 parts N per million dry soil.

Treatment	Nitrogen present as nitrate per million dry soil: Rothamsted Control (at start) 14·9: After 6 days	After 18 days	After 24 days	After 125 days	Woburn Control (at start) 16·5: After 6 days	After 18 days	After 24 days	After 125 days	Percentage of added N nitrified in Rothamsted soil: After 6 days	After 18 days	After 24 days	After 125 days
Control	15·3	12·5	13·0	15·5	15·6	19·8	20·2	27·7	—	—	—	—
Ammonium sulphate ...	28·0	37·4	41 5	45·8	34·3	48·0	44·8	45·8	45·2	77·6	91·7	100
1. Cyanamide	26·7	33·4	39 5	44 3	20·7	41·5	44·5	44·5	40·7	66·9	84·8	99·3
2. Cyanamide 3 parts, Dicyanodiamide 1 part	19·9	21·2	23 3	31·9	19 6	23 3	24·7	26·3	17·3	21·7	28·9	58·6
3. Cyanamide 1 part, Dicyanodiamide 3 parts	19 3	18 6	20·1	24 3	19·6	19 5	22·2	20 8	15·2	12·8	17·9	32·4

TABLE VI. *Effect of Dicyanodiamide on Nitrification of Cyanamide. Rothamsted Soil.*

N added: of cyanamide 50 mgs. N per kilo soil = 60 parts per million dry soil; of dicyanodiamide 15 mgs. N per kilo soil.

Treatment	N present as nitrate per million dry soil Control (at start) 15·9: After 6 days	After 18 days	After 42 days	After 162 days	N present as NH_3 per million dry soil: After 18 days	After 42 days	After 162 days	Millions of bacteria per gram fresh soil. Control (at start) 15·3: After 6 days	After 18 days	After 42 days	After 162 days
Control	21·6	21·5	25·6	31·6	4·0	5	1·0	14	17·9	19·0	8·7
Cyanamide alone	25·9	60·2	74·15	81·6	3·0	5	1·5	22	23·7	17·3	9·2
Cyanamide + dicyanodiamide (mixed prior to application) ...	20·0	19·9	28·4	56·3	23·5	26	16·8	11·5	16·3	17·0	8·0
Cyanamide + dicyanodiamide (added separately) ...	19·3	24·4	27·2	48·3	—	26	18·2	13·0	20·3	13·8	5·0
Cyanamide + dicyanodiamide (in same sample as nitrolim)	21·8	22·6	30·6	45·8	—	24	17·4	11·8	18·7	12·7	6·5

TABLE VII. *Effect of Dicyanodiamide on Nitrification of Ammonium Sulphate, Dried Blood and Cyanamide. Rothamsted Soil.*

Amount of N added: of ammonium sulphate, dried blood and cyanamide 50 mgs. N per kilo soil = 60 parts N per million dry soil; of dicyanodiamide 15 mgs. N per kilo soil.

Treatment	N as nitrate present per million dry soil. Control (at start) 8·8				N present as NH_3 per million dry soil. Control (at start) 2·6				Bacteria. Millions per gram fresh soil. Control (at start) 12·0			
	After 7 days	After 21 days	After 42 days	After 94 days	After 7 days	After 21 days	After 42 days	After 94 days	After 7 days	After 21 days	After 42 days	After 94 days
Control	12·4	18·8	17·5	20·6	3·8	1·3	3·9	5·2	11·0	14·1	8·3	20·0
Ammonium sulphate	19·5	33·7	41·4	55·0	28·5	16·8	16·5	7·9	15·0	9·8	13·3	14·7
Ammonium sulphate + dicyanodiamide	10·9	13 5	13·2	14·9	35·0	32·4	34·1	30·6	12·3	9·5	12·0	18·3
Dried blood	15·7	25·5	28·8	36·1	5·8	4·5	9·7	3·8	15·7	19·4	24·6	23·0
Dried blood + dicyanodiamide ...	10·4	12·5	14·7	13·3	9·1	17·4	20·5	18·2	10·4	15·2	24·0	19·2
Cyanamide	15·5	20·2	29 3	51·7	29·9	20·7	18·7	7·2	12·7	13·9	12·2	20·0
Cyanamide + dicyanodiamide ...	11·8	12·3	15·2	13·7	29·3	27·3	32 2	30·1	10·7	12·3	12·3	22·3
Dicyanodiamide (= 15 mgs. N per kilo soil)	9·1	8·5	13·3	11·7	3·9	3·2	5·2	3·9	11·0	8·3	13·7	14·0
Dicyanodiamide (= 45 mgs. N per kilo soil)	11·2	10·2	13·3	9·3	3·9	10 2	5·2	4·6	10·2	23·9	9·2	14·7

TABLE VIII. *Field Trials with Barley. Foster's Field, 1918. Previous Crop—Wheat.*

Treatment	Grain per acre, bushels		Straw per acre, cwt.		Total produce per acre, lbs.	
	Plot A	Plot B	Plot A	Plot B	Plot A	Plot B
Control (No N)	14·9	24·2	10·5	13·3	2000	2811
1. Ammonium sulphate ...	27·4	34·5	14·4	17·2	3078	3807
2. Cyanamide...	30·5	28·4	15·9	15·7	3435	3290
3. Cyanamide 9 parts Dicyanodiamide 1 part	29 2	34 2	15·5	16·6	3326	3712
4. Cyanamide 1 part Dicyanodiamide 9 parts	25·6	21·6	13·4	12·3	2898	2569

Amount of N applied in 1, 2, 3, 4 = that supplied in 1½ cwt. ammonium sulphate.

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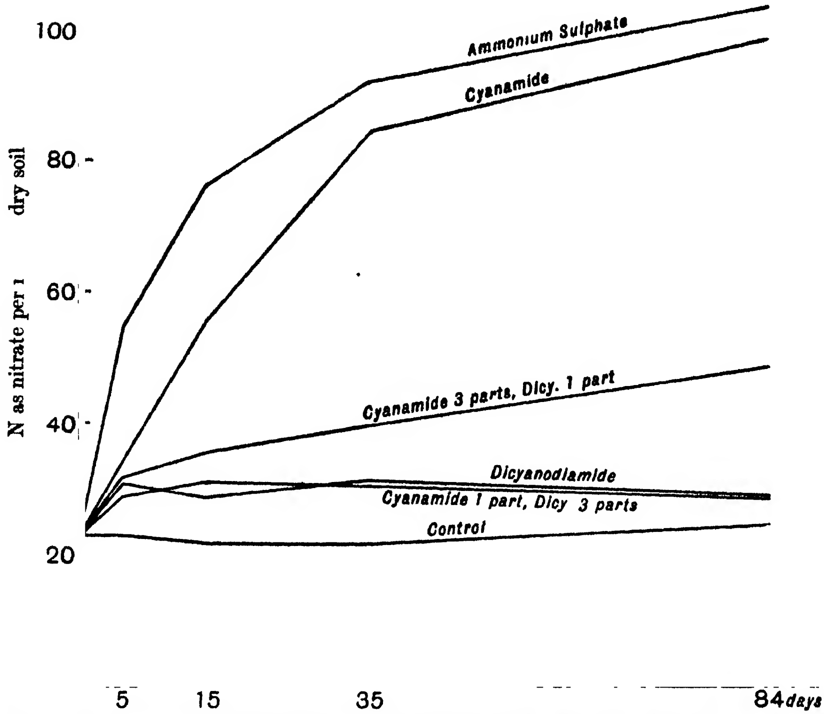


Fig. 1. Showing relative rates of nitrate production.

Ammonium sulphate	Control	Cyanamide	Cy. 3 Dicy. 1	Cy 1 Dicy. 3	Dicyano-diamide

Fig 2. Showing effects of various forms of nitrogen on growth of mustard. (N added = 50 mgs. per kilo soil.)

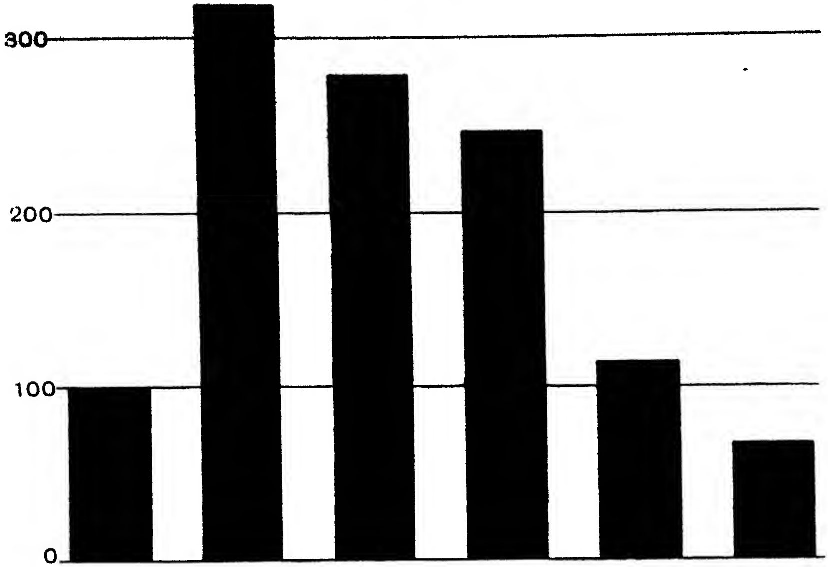
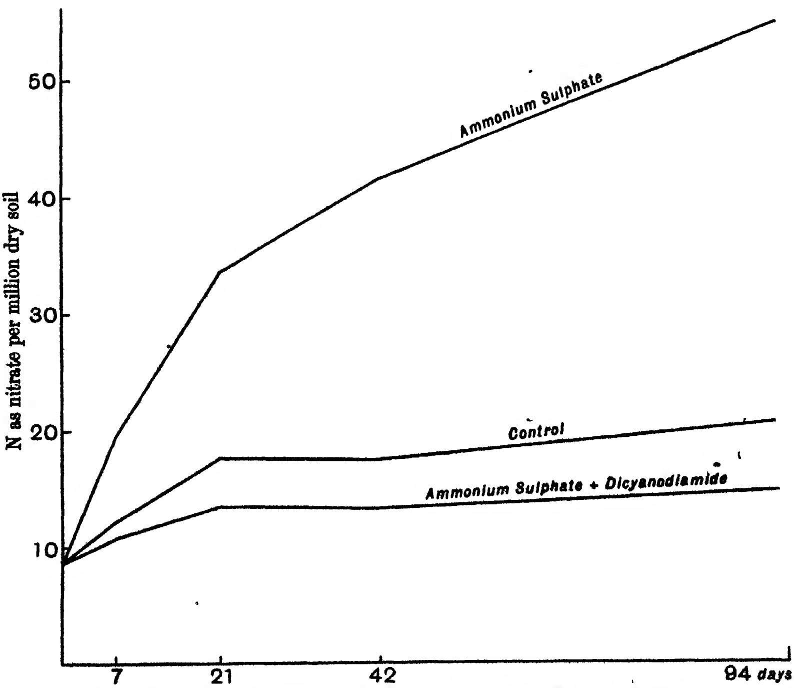


Fig. 2A. Showing relative weights of dry matter in mustard crop. (Control = 100.)



. 3. Showing depressing effect of dicyanodiamide on nitrification of ammonium sulphate.

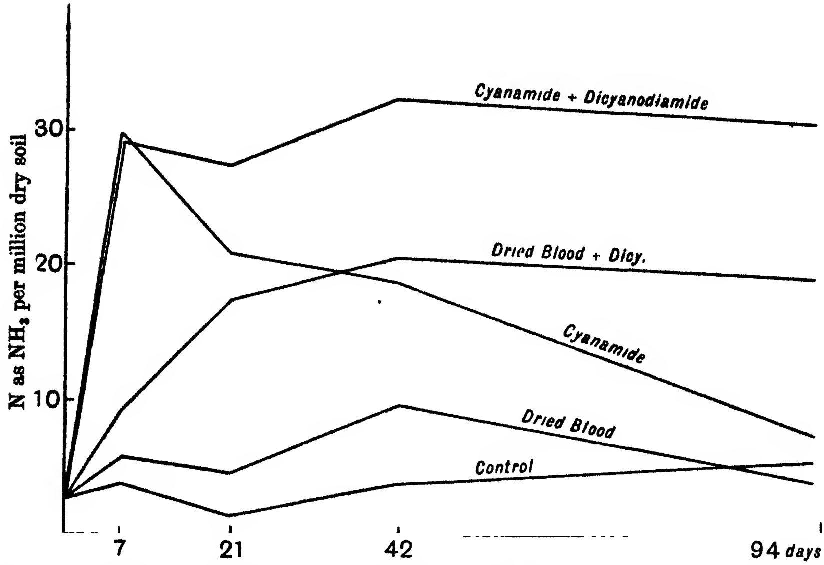


Fig. 4. Showing accumulation of ammonia from cyanamide and dried blood in presence of dicyanodiamide.

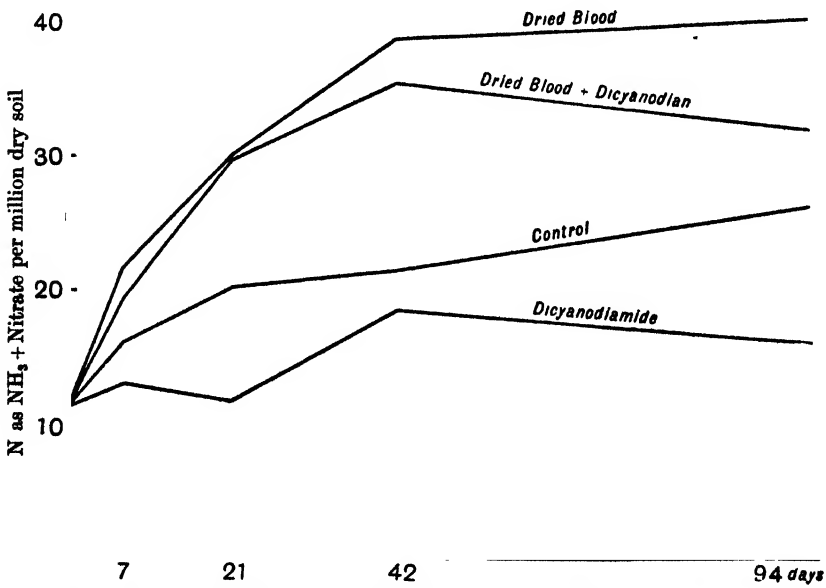


Fig. 5. Showing effect of dicyanodiamide on decomposition of dried blood.

(*Received August 3rd,* 1918.)

THE RANCIDITY OF PALM KERNEL AND OTHER FEEDING CAKES

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With the great increase in the use of palm kernel cake, which has taken place in this country in the last few years, attention has been drawn to the rancidity which the cake is liable to develop under certain conditions. R. B. Calder (see this *Journal*, April, 1916, vii, 470) has shown that the cake develops rancidity under sterile conditions and concludes that the action is due to the presence of a lipase. Calder has taken as his criteria of rancidity a sour smell in some cases and an acid reaction with litmus in others. There is some confusion in the use of the terms "rancidity" and "acidity." Although rancidity stands in close relationship to acidity and invariably accompanies a high acidity in oils and oil cakes, the terms are not synonymous. Lewkowitsch (*Oils, Fats and Waxes*, i, p. 50 *et seq.*) defines rancidity of an oil or fat as the production of a disagreeable smell and acrid taste, due to the direct oxidation of the free fatty acids by the simultaneous action of oxygen and light, and states that rancidity does not appear until free fatty acids are produced. Considerable doubt exists as to the nature of the oxidation products to which the rancid smell and acrid taste are due, some observers attributing these properties to aldehydes and ketones. In the absence of information as to the nature of the changes which occasion rancidity there is no method of estimating the extent to which rancidity has been produced in any particular case, though the acidity is an indication of the initial phase of the change. The degree of rancidity is influenced by the nature of the fatty acids present, that is to say, the ease with which they form oxidation products, and the odour and taste of the latter.

In the experiments upon which this paper is founded, and which have been carried out at the Imperial Institute, the amount of acidity developed under certain conditions in palm kernels and in cake and meal made from them, in comparison with other common feeding cakes, has been determined, and the nature of the action investigated. The results establish the presence of a fat-splitting enzyme or lipase in palm kernels

and in the manufactured cake and meal. The maximum lipolytic activities of the meal and cake are about one-half and one-third respectively that of the untreated kernels; while the activity of the kernels is fairly uniform, that of the meal and cake is irregular, indicating that a partial and uneven destruction of lipolytic activity has taken place during the manufacture of the latter products. Under suitable conditions, moisture being essential, the lipase acts on the fat liberating fatty acids with a consequent increase in the acidity of the cake. These experiments however have shown that this property is shared by other feeding cakes such as cotton seed, linseed, ground nut and coconut, and that palm kernel cake and meal develop less acidity than other cakes in the same time and under similar conditions.

Cotton seed and linseed cakes developed over four times as much acidity as palm kernel cake under equal conditions. The degree of acidity, however, cannot here be taken as a measure of rancidity, nor as an index to the palatability of the cake. On the other hand these experiments have shown that the nature of the liberated fatty acids must be taken into consideration in each case, and the explanation of the suggestion that palm kernel and coconut cakes, unlike other cakes, become rancid on keeping, lies in the fact that palm kernel and coconut oils contain glycerides of the lower fatty acids, which when hydrolysed by lipase yield volatile fatty acids, with unpleasant odours.

The fatty acids of palm kernel oil include 13 per cent. of the volatile fatty acids—caproic, caprylic and capric (see Lewkowitsch, *loc. cit.* II, p. 614), all of which have an unpleasant odour. Of these the chief is caprylic acid, which has an intense odour of sweat. Consequently the presence of a very small amount of these volatile acids in the cake, due to a slight decomposition of the oil, apart from any production of rancidity, is sufficient to impart to the cake an unpleasant smell. Further, palm kernel cake, even when freshly prepared, has a characteristic smell, probably due, in part at least, to a trace of these volatile fatty acids.

Coconut oil resembles palm kernel oil in composition, and also liberates caprylic acid, but the oils of linseed, cotton seed, and ground nut yield no volatile fatty acids on decomposition, or at the most mere traces, and are therefore not subject to this objection. Consequently cakes containing them are not liable to develop this peculiar odour on keeping, which is liable to be taken as an indication of rancidity.

No attempt has been made to ascertain whether the fatty acids of palm-kernel oil are more easily convertible into rancid bodies than the

fatty acids of other oil seed cakes. The results have however led to the conclusion that the objection to palm kernel cake lies in the initial production of fatty acids. On the other hand it has been shown definitely that palm kernel oil itself is not more readily decomposed into fatty acids than the oils of other feeding cakes investigated in these experiments.

Calder has stated that heating the moistened cake for half-an-hour at 70° C. would completely destroy the lipase and prevent rancidity of the cake from this source. The present investigation has shown that palm kernel lipase, whether in the kernels or in the cake and meal, is very resistant to heat, and capable of surviving to an appreciable extent four hours' exposure to a temperature of 97° C. in a moist state or two hours' exposure at 120° C. in a dry condition. To render the lipase in the cake inactive vigorous boiling with water for one hour was found to be necessary.

In view of the great resistance of the lipase to heat it is not practicable by this agency to render the lipase in the cake inactive before leaving the factory, and even if the lipase in the cake were rendered inactive the latter would be subject to deterioration by moulds and bacteria if allowed to become damp. It is therefore necessary in any case to preserve palm kernel cake and all other feeding cakes in a dry condition, and these experiments have shown that palm kernel cake may be kept for eighteen months in a dry condition without acidity or rancidity developing. At the end of this period the cake had not deteriorated, and was in sweet condition. On the other hand the most favourable conditions for the action of the lipase and the development of activity are warmth and excess of moisture.

EXPERIMENTAL.

Preliminary experiments showed that the amount of acidity developed by the cake was very small. It was therefore decided to investigate palm kernels themselves, in which greater activity was to be expected, in order to determine the conditions most favourable for the production of acidity.

The method of investigation consisted in maintaining the prepared mixture at a given temperature for a certain number of hours in a water bath and determining the rise in acidity. The mixture, to which 2 c.c. of toluene had been added to prevent bacterial action, was contained in a flask plugged with cotton wool. The meals were finely ground and the kernels reduced to a pulp by grinding. The mixture was thoroughly

shaken at frequent intervals, and in some cases the proportion of the components was adjusted to form an emulsion. Control experiments were performed to determine the increase in acidity due to the separate components of the mixture. The acidity of the mixture was determined by extracting first with warm water and then with alcohol and titrating the extracts with N/2 NaOH using phenolphthalein as an indicator. Owing to the presence of the natural colouring matter from the palm kernels the end point was not very sensitive and the experimental error amounted to $\pm$ 0·2 c.c. of N/2 NaOH.

The action of an emulsion of soya bean oil and palm kernels with or without the addition of acetic acid or water was first tried. Little or no acidity was produced under these conditions, though they were suitable for demonstrating the lipolytic activity of castor seeds, which were over a hundred times more active than the palm kernels. In these experiments the maximum activity of the palm kernels was developed in the absence of acetic acid and in the presence of water.

The next series of experiments confirmed this result and showed an excess of water to be necessary to the maximum development of acidity. These conditions however were not so suitable for the action of castor seed lipase, and palm kernels in these experiments had approximately one-sixth the activity of castor seeds. Neither the addition of acid nor of palm kernel fat increased the production of acidity.

The effect on their activity of previously heating the palm kernels was then investigated. Heating with water at 97° C. for 4 hours considerably reduced their activity but did not totally destroy it, and heating without the addition of water at 120° C. for 2 hours had a less destructive action. Total destruction of the power to develop acidity was effected by boiling the kernels with water for 3½ hours. Shorter periods were tried later in the case of the cake, and one hour's boiling was found to be sufficient. In these experiments the production of acidity was not due to (1) bacteria, since the operations were conducted under sterile conditions, (2) hydrolysis of the fat, since palm kernel fat in the presence of excess of water was not affected under the conditions of these experiments, (3) hydrolysis of other bodies in the kernels, since boiling palm kernels with excess of water for 3½ hours produced no rise in acidity, whereas if the acid producing action which takes place slowly at 35° C. was due to hydrolysis it should be accelerated by boiling. This behaviour points to the production of acidity as being due to the action of an enzyme. To confirm this view and determine whether the enzyme acts on the fat or on the other bodies present in the kernels a finely

ground fat-free powder was prepared from the palm kernels by repeated grinding and extraction with ether at room temperature, and allowed to act on soya bean oil and palm kernel fat under the necessary conditions. The action with soya bean oil was the more satisfactory and the results showed the prepared powder to possess fat-splitting properties, thus establishing the presence of a lipase in palm kernels. The activity of the prepared powder was low, a property which has been observed in the case of other lipolytic seeds, namely, that the activity diminishes on removing the fat. A fat-free powder treated with acetic acid according to Tanaka's method (see *J.S.C.I.* 1912, XXXI, 885) showed less activity than the above powder.

	Digestion		Acidity in c.c.s of N/2 NaOH		
Composition of reacting mixture	Time in hours	Temperature in °C.	Before digestion	After digestion	Increase
25 gms. of palm kernel cake + 50 c.c. of water	93	37	5·3	9·4	4·1
" " meal " "	93	37	5·7	11·05	5·35
25 gms. of p.k. cake + 100 c.c. of water previously heated at 97° C. for 4 hours, and then digested ...	163	37	5·3	6·85	1·55
20 gms. of p.k. cake previously heated dry at 120° C. for 2 hours; then 80 c.c. of water added, and digested ...	90	40	4·5	5·3	0·8
20 gms. of p.k. meal similarly treated ...	90	40	4·6	6·4	1·8
20 gms. p.k. cake previously boiled with 200 c.c. water for ½ hour ...	281	37	4·5	5·7	1·2
20 gms. p.k. cake previously boiled with 200 c.c. water for 1 hour ...	281	37	4·5	4·7	0·2
1 gm. of fat-free p.k. powder + soya bean oil + acetic acid ...	18	35	5·75	10·55	4·8
Soya bean oil + acetic acid ...	18	35	4·95	4·95	0·0
Fat-free p.k. powder + acetic acid ...	18	35	2·05	2·05	0·0
25 gms. of the following cakes + 100 c.c. of water:					
Palm kernel, second sample ...	235	39	5·4	12·7	7·3
Cotton seed, decorticated ...	235	39	5·0	59·0	54·0
" " undecorticated ...	235	39	5·7	33·5	27·8
Ground nut, decorticated ...	235	39	18·4	109·0	90·6
" " undecorticated ...	235	39	9·3	57·0	47·7
Coconut ...	235	39	25·0	38·0	13·0
Linseed ...	235	39	3·8	42·7	38·9

Having determined the best conditions for the action of palm kernel lipase, the cake and meal were then examined for lipolytic activity. Both developed acidity in a similar manner to the kernels, but the action was irregular and slower. Experiments at a room temperature of 17° C. showed that no appreciable action of the lipase takes place within a week

even in the presence of excess of water. A further set of experiments was carried out with six other oil-cakes under precisely the same conditions as for palm kernel cake. In each case there was a development of acidity, suggesting that the presence of a lipase is common to all oil-cakes. The amount of acidity produced was lowest in the case of the palm kernel cake.

In all cases experiments were repeated several times, for the sake of brevity, however, the general result only is given in the table (p. 141).

Conclusions.

Palm kernels and palm kernel cake and meal contain a lipase, which in the presence of moisture and warmth acts upon the oil present, liberating fatty acids, of which the volatile members have a strong sweat-like odour, and a very small amount of these acids is sufficient to impart to the cake a peculiar odour. This change does not occur if the cake is kept dry, a condition which is necessary to the preservation of all feeding cakes.

Freshly prepared palm kernel cake has a characteristic smell which is probably due to a trace of these acids produced during manufacture.

Palm kernel cake does not decompose more readily than cotton seed, linseed and ground nut cakes, these latter developing more acidity under similar conditions. These cakes differ from palm kernel cake in not yielding volatile fatty acids on decomposition. Palm kernel cake has been kept at the Imperial Institute for eighteen months and was at the end of the time in good fresh condition and showed no increase in acidity. The only precaution taken was to keep the cake dry.

(*Received June 22nd,* 1918.)

ON THE DECOMPOSITION OF CELLULOSE BY AN AEROBIC ORGANISM (*SPIROCHAETA CYTOPHAGA*, N. SP.).

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THE processes leading to the decomposition of cellulose and related substances in the soil present many features of considerable theoretical and economic interest. As is well known, cellulose displays a remarkable inactivity towards the simpler solvents, but under the action of strong acids it gives rise to the formation of less complex carbohydrates, sugars, such as cellobiose, dextrose, etc. On the other hand, certain organisms possess the faculty of attacking cellulose not only with great rapidity, but they also appear to do so with the formation of products which are not encountered in the purely chemical reactions. From the standpoint of soil fertility, the question acquires considerable importance in view of the enormous quantities of cellulose and its derivatives which, year by year, find their way into the soil in the form of plant residues and organic manures. As our acquaintance with the various soil processes extends it is becoming more and more evident that the inter-relations between carbon and nitrogen are not only exceedingly complex, but are also vital in their effect on the crop producing power of the soil.

The practical significance of some of these changes has already been discussed in an earlier paper[1] in which it was shown that by supplementing the amount of readily decomposable organic matter in the soil quite appreciable changes in the nitrogen content and in the fertility of the soil may be induced. In the present paper we propose to confine ourselves to the consideration of the processes whereby an inactive carbon

[1] Hutchinson, H. B., This *Journal*, 1918, 9, 92–111

compound—in the form of cellulose—becomes potentially active by its resolution into simpler products.

Although for many years the typical fermentation of cellulose was regarded as being an essentially anaerobic one, and due to the methane and hydrogen producing organisms described by Omelianski[1], it is nevertheless an indisputable fact that many of the changes in nature indicate the operation also of an aerobic process.

In this connection may be mentioned the known differences in behaviour of coarse sandy, and of clay, soils in their capacity to retain reserves of organic matter and humus. The former are reputedly "hungry," since decomposition changes proceed in them at the maximum. On the other hand, close textured or clayey soils present conditions which are less suitable for the degradation of organic matter. Apart from the physical constitution of the soil, the presence of excessive moisture, by the exclusion of air, also makes for less rapid decomposition; soils in districts of high rainfall, or those subject to flooding by surface springs, are generally characterised by a high content of undecomposed matter or crude humus. From the fact that cultivation and aeration of a soil lead to a reduction of organic matter, and that the recent work of Russell and Appleyard[2] shows that the composition of the free soil atmosphere differs only slightly from the normal, some direct relationship between decomposition and air supply may be deduced.

The first observations on the aerobic decomposition of cellulose appear to have been made by C. van Iterson[3], in Delft, who found that when a medium consisting of paper and mineral salts was distributed in shallow layer, inoculated with ditch mud, and incubated at 28°–35°, an energetic decomposition of the paper took place. In addition to the various organisms, sporogenous bacteria, spirilli and infusoria, that developed under these conditions, two others were encountered, the one being a very small rod-shaped organism, to which the name *Bacillus ferrugineus* was given, and the other a large micrococcus which was regarded as being unable to decompose the paper itself but which facilitated the decomposition in some manner not precisely indicated.

Decomposition of filter paper was further demonstrated by placing, in a glass dish, two pieces of Swedish filter paper between which some powdered ammonium magnesium phosphate had been introduced and

[1] Omelianski, W., *Compt. rend.* 1895, **121**, 653–655; 1897, **125**, 970–973, 1131–1133. *Arch. d. Sci. Biol.* 1899, **7**, 411–434. *Cent. Bakt. Par.* II. 1902, **8**.

[2] Russell, E. J. and Appleyard, A. This *Journal*, 1915, **7**, 1–48.

[3] Iterson, C. van, *Cent. Bakt. Par.* II 1904, **11**, 689–698.

moistened with a 0·05 per cent. solution of di-potassium hydrogen phosphate. The paper was infected by means of a suspension of garden soil, ditch mud, humus or dry leaves, and incubated at 24°–28°. After four to five days yellowish brown spots were produced on the paper which, at a later stage, became pulpy owing to the growth of the two organisms referred to above, until finally the individual fibres of the paper were enveloped in a "micrococcus mucilage." Van Iterson states, however, that decomposition of the paper could never be obtained by the use of such pure cultures as he was able to isolate from decomposing paper. His reference to the "micrococcus mucilage" encountered in crude cultures is interesting in its relation to our own results, which are given below. Van Iterson was also able to demonstrate the occurrence of a considerable number of filamentous fungi which are capable of utilising filter paper as a source of carbon.

At a later date Christensen[1] suggested the use of the cellulose decomposing power of a soil as an index of soil fertility. According to the amount of change undergone by strips of filter paper when reposing on the surface of different soils, the latter were placed in one of five grades – from 4 to 0 – of biological potency.

The first systematic investigations on aerobic cellulose decomposition were those initiated by Kellerman and McBeth[2] and subsequently continued by McBeth and Scales[3]. The former paid particular attention to the technique of cultivation and in this connection suggested the use of a number of special media such as cellulose agar, potato agar, starch agar, and an agar containing dextrose, mineral salts and ammonium sulphate. By the preliminary use of "elective" cultures, and plating out on the above media, they succeeded in isolating a number of organisms of which three, *Bacillus rossica*, *Bacillus amylolyticus* and *Bacterium flavigena*, were derived from cultures of anaerobic cellulose decomposing organisms obtained from Omelianski. McBeth and Scales examined soils from widely separated regions of the United States and isolated therefrom eleven species of bacteria while an additional four species were obtained from other sources. All these organisms are morphologically and physiologically distinct from Omelianski's hydrogen and methane organisms and grow well on ordinary gelatine media, although continued cultivation on such media is rapidly followed by loss of cellulose destroying power. None of these species produced gas

[1] Christensen, H. R., *Cent. Bakt. Par.* II. 1910, **27**, 449–451.

[2] Kellerman, K. F. and McBeth, I. G., *Ibid.* 1912, **34**, 485–494.

[3] McBeth, I. G. and Scales, F. M., U.S. Dep. Agr., Bureau Plant Ind., *Bull.* 266, 1913.

in peptone-carbohydrate broth, but the majority (four exceptions) formed more or less acid from sugars, starch and higher alcohols. With some species the principal by-products were found to consist of formic and acetic acids, while others only gave rise to traces of fatty acids. None of the solutions examined was found to contain any trace of aldehydes, ketones, alcohols, or of carbohydrates capable of reducing Fehling's solution.

Concurrently with these American investigations, the question of cellulose fermentation had been taken up by Löhnis and Lockhead[1], who employed an agar medium consisting of mineral salts, sodium nitrate and 0·3–0·5 per cent. chemically pure cellulose. The paper partakes of the nature of a preliminary communication; so far as we are aware, a description of the causative organisms of fermentation has not been published.

EXPERIMENTAL.

Preliminary experiments to demonstrate the presence of aerobic cellulose decomposing organisms in Rothamsted soils presented practically no difficulty, the usual procedure being to prepare flat bottomed 300 c.c. flasks containing 100 c.c. of mineral salt solution[2], 0·25 grm. sodium nitrate, and 1·0 grm. filter paper. Before sterilisation, the latter was orientated so that it reposed in direct contact with the sides of the flask, with its upper portion protruding above the level of the culture liquid. Inoculation of these flasks was carried out by the introduction of about one gram of field or garden soil. After incubation at 25° for upwards of 4–6 days, the filter paper at, or slightly above, the level of the liquid assumed a yellowish or yellowish-brown colour and gradually lost its consistency. Although it was possible to reproduce this change repeatedly by transference of some of the decomposing paper to further flasks, such elective culture failed to yield a culture which possessed any high degree of purity, and it was decided, therefore, to attempt some method of plating out.

Prior to the publication of Kellerman and McBeth's paper, two methods were in general use. The first of these consisted in the use of an agar medium composed of mineral salt solution, with 0·25 per cent.

[1] Löhnis, F. and Lockhead, G., *Cent. Bakt. Par.* II. 1913, 37, 490–492.

[2] The stock mineral salt solution used was the nitrogen-free solution given by Meyer (*Practicum d. botan. Bakterienkunde*, Jena, 1903, p. 15) and had the following composition: 1·0 grm. KH_2PO_4, 0·1 grm. $CaCl_2$, 0·3 grm. $MgSO_4+7H_2O$, 0·1 grm. NaCl, 0·01 grm. Fe_2Cl_6, 1000 grm. H_2O.

sodium nitrate and 1·5 per cent. agar; this stock agar was tubed in portions of 10 c.c. and sterilised in the autoclave. A number of such sterile portions were then poured into a like number of Petri dishes and, after consolidation of the medium, a circular piece of sterile filter paper was laid and pressed upon the surface of the agar. Fresh portions of the melted agar were then inoculated with various dilutions of the culture to be plated out and were then poured in as thin a layer as possible over the surface of the paper. In this manner plates were obtained which on incubation for upwards of a week showed quite strong bacterial growth, the paper became covered with bright yellow spots (colonies), and eventually became transparent owing to the complete dissolution of the paper (Plate I, fig. 1).

Although otherwise satisfactory growth of the causative organisms could be obtained in this manner, this method of preparing plate cultures was abandoned; in the first place, because the colonies uniformly gave what were regarded as impure growths, and, secondly, on account of the fact that the colonies resulting from the inoculation of any one plate appeared to bear little relation to the number of organisms actually used as inoculant.

The second method consisted in the preparation of a suspension of cellulose by grinding up filter paper with coarse sand and water. By this means a quantity of finely divided cellulose was obtained and made into agar similar in composition to the preceding one. The preparation of cultures with this medium gave, after upwards of 8–10 days, plates showing a number of zones in which, by the growth of the organism, the cellulose had become partially or wholly dissolved (Plate I, fig. 2). Microscopical examination of plates from crude cultures revealed the presence of numerous surface and embedded colonies which, however, had no obvious connection with the zones of change, and which on transference to filter paper tubes did not result in characteristic breakdown of the paper. Microscopical examination of the agar of the zones was made and showed the presence of two chief cell forms which have also been invariably found in cultures capable of inducing active and typical decomposition. One of these forms, usually referred to as the "thread" form, was found in abundance in young cultures on agar or on paper, and consisted of a fairly long thin filamentous and frequently curved cell, that was stained with difficulty by ordinary methods. The second form was that of a large "coccus" which, particularly in old cultures, occupied a predominant position.

From time to time we were able to obtain colonies on cellulose agar

plates which, under the microscope, appeared to consist wholly of the filamentous organism, but inoculation to fresh medium and incubation for a few days resulted, without exception, in the incidence of the coccus form.

Despite the preparation of a considerable number of subcultures and the use of widely different media[1], we have been unable to effect a permanent separation of these two forms, nor has the employment of the media suggested by Kellerman and McBeth, whose paper appeared about this time, been followed by any greater measure of success.

Various attempts were made to set up conditions which might conceivably favour one of the forms at the expense of the other. Experiments were carried out, for example, in which tubes containing filter paper and mineral salts were inoculated with a culture of the two forms and then subjected to a range of temperatures varying by 2° from 40° to 62°. After seven days, growth occurred in all tubes subjected to temperatures of 40°–56° for ten minutes, while after nine days growth was also evident in the tube heated to 58°. None of the tubes showed a pure culture either of the thread or coccus form.

Similarly, attempts to obtain simplification of the flora by differential reaction of the media yielded negative results. Treatment of crude cultures with volatile antiseptics was equally ineffective in bringing about separation of the two forms. Chloroform, toluene, and carbon bisulphide all had the effect of destroying both the filamentous and the coccus forms. On the assumption that, on plating out, simple coherence of the two forms occurred, mechanical dissemination of the cultures by means of an atomiser and exposure of cellulose agar plates to the spray was tried. In this manner we obtained characteristic colonies, but none of these was found to be pure. We were, therefore, inclined to regard the mutual relationship of these two forms as strictly symbiotic -- neither of the forms being able to grow in the absence of the other. It was obviously impossible under these conditions to ascertain which of the forms was the one actually responsible for the observed changes in the filter paper, and the investigation was, consequently, temporarily abandoned.

[1] The media tested and found unsuitable for the growth of the cellulose decomposing organisms, or which failed to allow of separation of the two forms, include nutrient gelatine and nutrient agar with and without dextrose, sodium nitrate-mineral salt-cellulose agar, sodium nitrate-dextrose agar, soil extract-cellulose agar, with the addition of 1·0 per cent. of the following sources of nitrogen—asparagin, ammonium citrate, peptone, sodium ammonium phosphate, potassium nitrate: Kellerman and McBeth's cellulose agar, dextrose agar, starch agar, and the numerous solutions suggested by Meyer (*loc. cit.* p. 24) for diagnostical purposes.

Subsequent work with cellulose agar plates resulted in the production of colonies which agreed macroscopically with those previously obtained, but microscopically appeared to consist solely of the "thread" form. Inoculation of cellulose agar slant tubes and incubation for five days was, however, followed by the occurrence of the coccus form again. Hence it was assumed that isolated cells of the latter form had eluded search under the microscope.

Having occasion at a later date (1915) to prepare tubes and plates of the so-called mixed culture, it was evident that growth in liquid culture with filter paper was not only more vigorous but arose in much less time than was the case on agar plates. It was accordingly decided to prepare subcultures by the dilution method, and to this end a set of test tubes was prepared, each tube containing a piece of filter paper 15×60 mm. in size, and 5 c.c. of stock mineral salt solution containing 2·0 grm. of sodium ammonium phosphate per litre. In this as in all subsequent sets the reaction of the mineral salt solution was first brought to the neutral point with phenolphthalein and then to each 100 c.c. of solution 1·0 c.c. of N/10 sodium hydroxide solution was added.

The requisite attenuations were made in physiological salt solution and ranged down to 1×10^9. By means of sterile pipettes one drop ($\frac{1}{20}$th c.c.) of a particular attenuation was introduced into each of six tubes set aside to receive this attenuation.

After incubation for four days at 30°, growth was evident in all tubes which received attenuations up to 1×10^7. Of the six tubes receiving the next higher dilution, viz. 25–30 inclusive, tubes 26, 27, 28 and 29 showed the characteristic colour and growth on the filter paper, while examination of tube 29 showed it to contain what was apparently a pure growth of the thread form. As an alternative to incubating the culture for a further period, thus giving opportunity for the development of any occasional cell of the coccus form that might be present, it was decided to prepare a fresh dilution set on similar lines. This was completed in less than an hour and, in addition, the culture was transferred to ordinary filter paper tubes. Incubation for a further period of four days served to induce growth in the dilution tubes as high as tube 23 but it was then evident on microscopical examination that all the tubes contained a mixture of the "thread" and "coccus" forms. In view of the fact that the inoculant used for this set of cultures contained apparently a pure thread form and could most certainly not have contained anything approaching the number of coccus forms indicated by this second dilution set, viz. 40,000,000 per c.c., the conclusion was

unavoidable that there existed a vital and intimate connection between the two forms. Subsequent dilution cultures as well as direct examination under the microscope confirmed this view, and resulted in observations which are given in detail below. On account of the peculiar developmental cycle through which the organisms appear to pass, as well as on other grounds, we are unable to regard it as a representative of the true Bacteriaceae. In form (especially when grown in liquid media), lack of flagella, and perfect flexibility of cell, it approaches more closely to the Spirochaetoideae and we suggest therefore the name *Spirochaeta cytophaga*. While doing so we recognise that the organism under consideration exhibits a number of features which have not hitherto been observed in the spirochaetes, features which however appear to indicate a more complex development than that of the true bacteria.

Morphology of *Spirochaeta cytophaga*.

Young cultures of *S. cytophaga* invariably exhibit the predominance of long thin frequently incurved cells, which consequently do not permit of measurement with any degree of accuracy. In stained preparations the mean dimensions of the cells have been found to lie in the region of 3μ along the major axis, while the mean diameter is from 0·3–0·4μ. Under certain cultural conditions extremely long twisted filaments are obtained, the length of which may extend to upwards of 40μ. The form assumed by these filaments depends largely on the method of fixing which is adopted; if the smears are allowed to dry at room temperature before being fixed in the flame the majority of the cells will be found to be much curved and to have taken up the shepherd's crook, **S** and **U** forms shown in Plate I, fig. 3. If, on the other hand, the film be dried by a short exposure to the flame the filaments are as a rule less bent and are frequently perfectly straight. Cultivation of the organism in liquids in which the cellulose is completely immersed appears to induce the highly undulating sinuous form shown in Plate I, fig. 4.

In older cultures, a predominant position is occupied by the ovoid or spherical form, the dimensions of which are approximately 1·5μ diameter. Owing to the fact that this form cannot be regarded any longer as an infection form it is considered desirable to abandon the use of the term "coccus," which might conceivably lead to a certain amount of confusion. Our observations show that this spherical form possesses little analogy with the true bacterial spore while, on the other hand, the use of the term "cyst" might imply more than is perhaps warrantable

at the present time. We therefore propose to refer to it for convenience as a "sporoid" stage until more information respecting the affinity of this to other organisms is obtained.

Motility. Evidence of independent movement of the filamentous form may be obtained from observation in hanging drop, although care must be taken to avoid appreciable changes in temperature. Organisms which are attached to portions of cellulose fibre display a slow rotatory movement, while occasional cells proceed with a sinuous action through the culture liquid. Occasionally, on the extreme edges of the drop the central portion of the filament remains stationary but the ends are reflexed so that, according as to whether they move in the same or opposite directions from the major axis, the outline of the cell assumes an **O** or **S** shape. The filamentous form, therefore, possesses perfect flexibility. Up to the present, we have been unable to demonstrate the presence of flagella.

Staining reactions. S. cytophaga generally takes up the conventional bacteriological stains with comparative tardiness. Methylene blue, while failing to give any satisfactory result with the filament form, gives faint staining of the sporoids. The organism reacts negatively towards Gram's stain and does not respond well to the action of dilute fuchsin. The most satisfactory results obtained hitherto have been by the use of hot carbol fuchsin, for upwards of a minute, without subsequent differential treatment. Bold preparations may also be secured by a "deposition" stain, i.e. either flooding the film with weak alcoholic fuchsin, and after tilting the slide to remove the superfluous stain, allowing the remaining solution to dry on the film or, alternatively, by placing a cover-glass on the film, flooding the intermediate space with the stain, and then quickly raising the cover-glass from one side. By this means small quantities of solution are left in the immediate vicinity of the cells, on which the residual stain is ultimately deposited. The staining capacity of the organism varies greatly, however, with the cultural conditions and occasionally even the most intensive methods fail to yield satisfactory results.

Spore formation. Spore formation in the ordinary sense of the term, i.e. the production of a stage which possesses greater powers of resistance to external factors, does not appear to take place with *S. cytophaga.* Any other interpretation of the function of a spore as, for example, the contention that spore production is nothing more or less than the adoption of a resting stage, is difficult of application to the organism under consideration. Although there is abundant evidence that the

filamentous form predominates in the early stages of growth, and the spherical form occurs more abundantly in older cultures, it is quite unjustifiable to deduce from this that the "sporoid" constitutes a resting stage or that it is in any degree physiologically less active than the earlier form.

Aerobism. S. cytophaga is essentially aerobic in character, and practically the only decomposition of cellulose occurs at or slightly above the level of the culture solution. In those cases where the cellulose sinks to the bottom of a comparatively shallow layer of liquid as, for instance, occurs with culture solution containing precipitated cellulose, growth proceeds with extreme slowness.

Developmental cycle of S. cytophaga. The construction of the developmental cycle of *S. cytophaga* is associated with difficulties which up to the present we have not been able entirely to overcome. In the first place, the most satisfactory method of procedure is admittedly the adoption of the hanging-drop culture method in which continuous observations of the development of any specific organism may be made. Unfortunately, and in spite of a considerable amount of work with a large number of media, we are still without a synthetic medium on which the organisms can be well isolated and which permits of growth observations. The use of cellulose, either as fibre or in the form of a precipitate, is obviously unsuitable on account of physical interference with the image of the organism. From time to time attempts have been made to employ the Indian ink method, but without any degree of success. On the other hand the diminutive size not so much of the cell itself but of the cell structures— which apparently undergo a series of changes— renders it difficult even with the use of the highest magnifications to obtain any definite idea of the exact sequence of the changes which proceed in active cultures.

Under these circumstances the cycle of development outlined below is only submitted tentatively and is therefore subject to such revision as future experience may indicate. As far as possible photomicrographs have been secured of typical intermediate stages between the thread and the sporoid forms and some of these are given on Plates I–III. It may also be added that in those instances where a particular cell exhibits noteworthy features, the photographs are so arranged that such cells are below the centre and in line with the vertical axis of the plate.

Two types of growth of *S. cytophaga* appear to be distinguishable. The first may be termed the direct, or purely vegetative, growth whereby new cells are formed by transverse fission, and which proceeds extensively

in young cultures. The second may be referred to as indirect and possibly generative growth in which both cell and cell contents appear to undergo a definite series of changes.

The predominating form in young cultures will generally be found to consist of simple, sinuous filaments the length of which is about ten times the diameter. Carbol fuchsin is fairly readily and uniformly taken up and shows the slightly tapering ends of the cell. With increasing age there occur a number of cells which begin to show differentiation of the cell structures, the terminals becoming less intensely stained, while the cell contents assume the form of a densely stained equatorial band (Plate I, fig. 6) or may take up the spherical form (Plate II, figs. 2 and 5). The nuclear substance then presumably takes up a transverse position and finally undergoes division which is accompanied by a constriction of the cell wall. During the latter phases the cell itself has become appreciably thicker and less filamentous, while owing to almost complete indifference to the action of the stain, the cell wall is perceptible as little more than a "shadow" form (Fig. 1 below). After further constriction the two halves of the cell apparently become detached, the resultant oval or ovoid cells each containing a plate or disc of nuclear substance which remains attached to the cell wall when the spherical or sporoid stage is eventually reached. Finally, the sporoid—which up to this has exhibited comparatively slight staining capacity—becomes evenly and intensely stained, presumably owing to a dispersion of nuclear substance, and gives rise to the thread form. This, therefore, must be accepted as being merely our interpretation of the differences of cell form and cell contents which may be observed in stained preparations of cultures of different ages. It is, of course, fully recognised that heat fixation is objectionable on account of the risk of distortion of nuclear figures or of chromatin substance, and that all structures which take up fuchsin are not necessarily of the nature of nuclear substance. It may be mentioned, however, that identical appearances are to be obtained by formaldehyde-alcohol fixation and the use of stains such as those of Leishman and Geimsa.

In the figure on p. 154 the main intermediate stages between the filamentous and the sporoid forms of *S. cytophaga* are diagrammatically represented, the most frequently occurring types being arranged in horizontal order from left to right, while a few of many divergent examples are grouped both above and below.

Of these variations from the normal may be mentioned the tendency towards the production of highly sinuous forms in liquid media, while

cultivation under unfavourable conditions is frequently followed by intense granulation of the thread form. This is evident, for example, in cultures containing an excess of nitrogen or, in fact, of soluble organic substances generally—nutrient beef agar, 1·0 per cent. peptone, 0·5 per cent. asparagin, and also in dilute solutions of phenol, etc. The most pronounced changes of this type were observed in cultures which, in error, had been subjected to a temperature of 36°–37° for some days. The culture that actually underwent this exposure was characterised by low stain receptivity, the majority of the cells showing phases such as are represented in Plate II, figs. 2 and 3. On transference of this

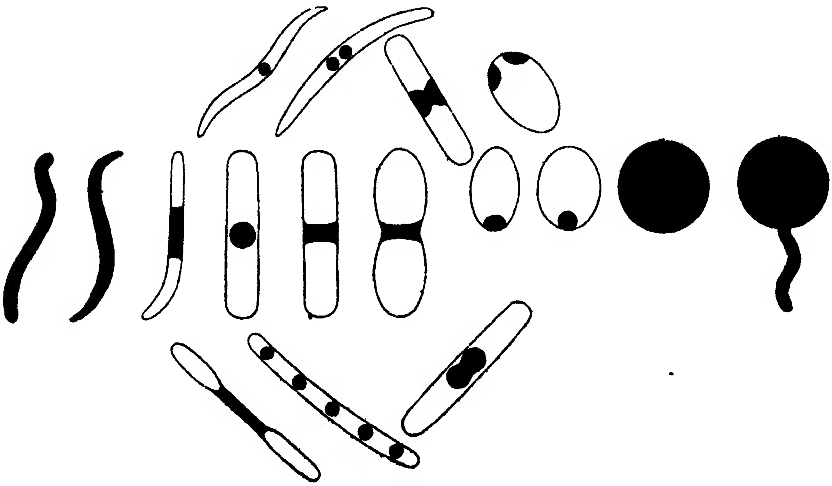

Fig. 1.

culture to a lower temperature (25°) and especially on inoculation to fresh medium, almost all the filamentous forms exhibited strong granulation, and this characteristic persisted for at least two generations after the culture had ceased to be under unfavourable conditions (Plate III, fig. 6). Well-marked granulation is of interest morphologically, in that it imparts to the cell an appearance somewhat similar to that found by Leishman[1] to be possessed by spirochaetes giving rise to the formation of "coccoid granules." There is no evidence however that these granules of *Spirochaeta cytophaga* have any special significance.

A further divergence from the normal course appears to be the formation of a pre-sporoid stage possessing either double granules or a band of nuclear substance (Plate II, figs. 3 and 4). The general for-

[1] Leishman, W. B., *Trans. Soc. Trop. Med. Hyg.* 1910, 3, 97.

mation and persistence of sporoids tend to become accentuated with increasing age of the culture until, at the end of two to three weeks, the whole of the bacterial mass and also of the partially decomposed cellulose fibres appear to consist exclusively of micrococci. This was, no doubt, the phase observed by van Iterson, who refers to the fibres as being embedded in "micrococcus mucilage."

Until the organism has been subjected to further study by the use of special cytological methods it would be premature to express any opinion as to the significance of the changes which the organism undergoes. It is hoped that sufficient evidence has been already adduced to indicate the complexity of these changes.

PHYSIOLOGICAL.

On account of the difficulty of separating the filamentous and the sporoid forms of the organism and, incidentally, of establishing their relationship to one another, we recognised at an early date the desirability of obtaining some synthetic medium suitable for the preparation of hanging-drop cultures and on which continuous observations of the organisms would be possible. With this end in view it was decided to investigate systematically the relative value of various forms of nitrogen and carbon.

As is shown by the results of the experiments reported below, *Spirochaeta cytophaga* is markedly specific in its nutritive requirements; at the same time it displays extraordinary sensitiveness towards the presence of compounds which it is apparently unable to utilise but which with the general run of bacteria have been found to serve as sources of nitrogen or carbon or both peptone, amino-acids, sugars, salts of organic acids, etc. Since the organism invariably grows well in mineral salt solution with cellulose, the general method adopted in systematic tests was to prepare a range of test tubes having the same basal mineral salt solution, but with different, and sometimes increasing, amounts of carbon or nitrogen compounds. According as to whether cellulose was also present or absent, it was possible to ascertain (*a*) inhibitive effects, and (*b*) nutritive values of the compound tested.

In the summary of these experiments recourse is had to tabular representation of the results; the individual cultures are arranged as a rule in order of gradually increasing concentration, while the amount of growth or, where present, the destruction of cellulose is indicated as $-$ = lacking, $-+$ = very faint, $+$ = distinct, $++$ = fairly strong, and $+++$ = vigorous.

Relations to Sources of Nitrogen.

Early attempts to obtain pure cultures of *Spirochaeta cytophaga* by means of the conventional media sufficed to demonstrate the unsuitability of such media as nutrient agar and nutrient gelatine with and without the addition of dextrose. The invariable result was that while any organism resembling *S. cytophaga* failed to grow, various contaminating forms were secured which, on transference to cellulose media, were found to be incapable of bringing about any dissolution. The absence of cellulose from these media might, of course, have contributed materially to these negative results, but later experience indicated that ack of growth was more probably attributable to (*a*) the concentration of soluble compounds and (*b*) the unsuitability of the more complex nitrogen compounds as sources of nitrogen.

Although the use of nutrient agar had, therefore, to be abandoned, various attempts have been made to induce growth of the organisms on this medium, and in this respect the following may be mentioned. Negative results were obtained when agar plate cultures were made from dilutions of 1/200, 1/40,000 and 1/4,000,000 of active culture, the organisms being evenly distributed in the usual manner. Equally bad results followed the transference of *loopfuls* of active culture to the surface of nutrient agar plates. When *masses* of decomposing filter paper were transferred to the surface of nutrient agar plates, slow growth of the organism or, at least, continued enzymic action was evident. The paper was gradually resolved and gave rise to a semi-translucent bacterial mass, yellow in colour and of mucilaginous consistency. The bacterial filaments became highly granular and apparently suffered in vitality. Transference of portions of this bacterial mass to fresh nutrient agar was not followed by growth, thus showing that the capacity of growth without cellulose had not been acquired.

The relations of the organism towards peptone have been investigated and the results are given in Tables I and II. In the preliminary set (Table I) a short range of concentrations was tested, in all of which growth occurred with the exception of 1·0 per cent. solution. As it was possible that this solution had not received its proper amount of inoculant, several reinoculations were made with active culture from the next lower concentration but these also failed to give rise to growth. There was thus no acclimatisation to higher concentrations.

The second series included a set of tubes without cellulose to test the capacity of the organism for growth on peptone.

TABLE I.

Growth of S. cytophaga *in Peptone Solution with Cellulose.*

	Concentration of Peptone Solution (per cent.)				
	0·0001	0·001	0·01	0·1	1·0
Growth ...	+ +	+ +	+ +	+ +	

TABLE II.

Growth of S. cytophaga *in Peptone Solution with and without Cellulose.*

Growth after 8 days	Concentration of Peptone Solution (per cent.)								
	–	0·005	0·010	0·025	0·050	0·10	0·25	0·50	1·0
Without cellulose	–	–	–	–	–	–	–	–	–
With cellulose and ammonium salts	+ + + +	+ + + +	+ + + +	+ + + +	+ + +	+	+	–	–

The unsuitability of peptone as the sole nutrient is well brought out, the organism failing to show growth in any of the various concentrations tested. In the presence of filter paper strong growth occurred up to the 0·025 per cent., but marked inhibition set in with 0·25 per cent. solution.

Comparison of simpler nitrogen compounds. Throughout the cultural work in connection with *S. cytophaga* the superior value of inorganic forms of nitrogen has been well demonstrated, and a comparison of these is afforded by Table III. Proceeding from a basis of normal mineral salt solution and filter paper, tests were made with increasing amounts of sodium ammonium phosphate, ammonium phosphate, sodium nitrite and sodium nitrate.

TABLE III.

The Relative Value of Inorganic Nitrogen Compounds.

Growth after 6 days	Concentration of salt solution (per cent.)									
	0·004	0·009	0·018	0·037	0·075	0·15	0·31	0·62	1·25	2·50
Sod. Amm. Phosph.		–	– +	+	+ +	+ +	+ +	+ +	+	–
Amm. Phosph.		– +	+	+ +	+ +	+ +	+ +	+	–	–
Sod. Nitrite	– +	+	+	+	+ +	+	+	–		–
Sod. Nitrate	–	+	+ +	+ +	+ +	+ +	+ +	+ +	+	–

The results obtained after three days' incubation were on the whole similar to those given above, except that with longer incubation the growth was stronger and that the critical concentration showed a general displacement to the higher levels. It may be assumed that the

lower limits of growth are probably associated with minimum nitrogen requirements, since the more highly nitrogenous salts give better growth than sodium ammonium phosphate. On the other hand, the upper limits of growth do not appear to be determined by nitrogen concentration, since sodium nitrate gives as good results as sodium ammonium phosphate, although it contains about 2½ times as much nitrogen. It is also to be expected that the favourable effect of sodium nitrate is a complex one, i.e. with increasing abstraction of nitrogen the reaction of the solution would be better maintained owing to the formation of sodium carbonate or of neutral salts of acid by-products.

A supplementary set of experiments included the use of a number of the simpler organic nitrogen compounds which, from their constitution, might be expected to serve as sources of nitrogen. Two concentrations were tested, viz. 0·5 and 0·05 per cent., the general method being to add the test solution to the slightly alkaline mineral salt solution after sterilisation, thus reducing the risk of decomposition of the compound. The results are given below (Table IV).

The majority of the compounds are thus suitable for growth, although their values are widely different. The behaviour in urea is peculiar and requires further investigation, but from the paucity of growth in the initial stages and the abundance of growth after 40 days it might be assumed that acclimatisation to the compound took place, or that the power of hydrolysing the urea was eventually acquired. The results with hydroxylamine sulphate and hydrazine sulphate are in conformity with the frequently observed intolerance of *S. cytophaga* of any reducing substances. Acetamide and asparagin give poor results in low concentration but fairly good effects when present to the extent of 0·5 per cent. Formamide, on the other hand, is apparently of value only in low concentrations (0·05 per cent.), and acts inhibitively in strengths approaching 0·5 per cent.

Relations to various Sources of Carbon.

The carbon requirements of the constituent species of the soil flora may be met by a considerable range of compounds—from carbon dioxide through the paraffins, alcohols, fatty and hydroxy-acids, and the various monoses and bioses. Of these, such compounds as glycerol and mannite, malic, citric, succinic and tartaric acids, dextrose, maltose and saccharose are of the most general utility. At the same time several of the species exhibit a high degree of specificity as, for example, the nitrifying organisms and some of the sulphur bacteria. On this account, and in

TABLE IV. *The Relative Value of some Simple Organic Nitrogen Compounds.*

Time	Concentration	Sod. Amm. Phosphate	Ammonium tartrate	Hydrazine sulphate	Hydroxylamine sulphate	Sodium nitrite	Ethyl nitrate	Formamide	Acetamide	Succinamide	Carbamide	Asparagin	Tyrosin
5 days	0·05 %	+	+	−	−	−	− −	− +	−	−	−	− +	−
	0·50 %	−	−	−	−	+	−	−	+	+	−	+ +	−
10 days	0·05 %	+ + +	+ +	−	−	+ + −	− +	+	−	+	−	− +	−
	0·50 %	− +	−	−	−	+ −	+	−	−	+ +	−	+ + +	−
15 days	0·05 %	− + +	− +	−	−	+ + +	− +	− +	−	+	−	− +	−
	0·50 %	− + +	−	−	−	+ +	+ +	−	+	− +	−	+ + −	−
40 days	0·05 %	+ + +	+ +	−	−	+ + + +	− +	− + + +	−	+ + +	− +	+	−
	0·50 %	+ − +	+ +	−	−	+ − − +	+ +	−	−	− +	− + + +	+ + + +	−

TABLE V. *The Relative Effects of Carbohydrates, etc. on Growth of* S. cytophaga.

Growth after 12 days	Concentration	Mannite	Dulcite	Adonite	Arabinose	Dextrose	Levulose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Starch	Dextrin	Inulin
Without cellulose	0·10 %	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1·00 %	−	−	−	−	−	−	−	−	−	−	−	−	−	−
With cellulose	0·10 %	+	+	+	−	−	−	−	+	−	− +	+	+	+	+
	1·00 %	+	+	+	−	−	−	−	+	−	−	+	+	− +	+

view of the lack of success with nutrient dextrose agar and with dextrose mineral salt agar, it was considered desirable to ascertain what position *S. cytophaga* would assume in this respect.

The first series of experiments included a range of higher alcohols and carbohydrates some of which have been found useful for general diagnostical work and possess a high nutritive value for bacterial growth. Tests were made in the first instance with two concentrations, viz. 0·1 and 1·0 per cent. Cultures were made with and without cellulose in order to give (*a*) inhibitive, and (*b*) nutritive effects. The results of this series are given in Table V.

From these, a number of interesting points emerge. Of the fourteen compounds tested, none appears to be capable of meeting the carbon requirements of the organism either in 0·1 or 1·0 per cent. concentration. While unsuitable for growth, they evidently differ greatly in inhibitory power, since decomposition of the cellulose proceeded normally in some, but was completely absent in others. This disparity which, at first sight, might appear to be somewhat inexplicable, permits of an explanation on the basis of the chemical behaviour of the various compounds. All those compounds which possess reducing properties, e.g. which induce reduction in Fehling's solution, are shown to exert marked inhibitory effects on the organism. The behaviour towards carbohydrates is, therefore, similar to that previously observed with nitrite, hydrazine and hydroxylamine. Whether this effect is directly connected with an interference of the aerobic requirements of the organisms or of some specific enzymic change must be left open, but it may be mentioned that the contrary effect—stimulation of the anaerobic organisms—has been observed on the addition of such reducing substances as dextrose, sodium formate, pyrocatechin, sodium hyposulphite, etc. to nutrient media[1].

In our experiments saccharose, raffinose, starch, inulin and the higher alcohols are non-toxic even in 1·0 per cent. solution; subsequent tests with gum arabic gave the same result. Dextrine is toxic in high, but non-toxic in low, concentrations, while all the rest with the exception of lactose cause inhibition even in 0·1 per cent. strength. Since the two concentrations chosen for the experiment were more or less arbitrary, two further tests were carried out. The first consisted in the use of dextrose in the presence of cellulose and indicates that inhibition occurs when the concentration exceeds 0·05 per cent.

[1] Kitasato, S. and Weyl, T. *Zeitsch. Hyg.* 1890, 8, 41. Beijerinck, M. W., *Verhand. d. konink. Akad. Wetensch.* Amsterdam, 1893.

TABLE VI.

Inhibitory Effect of Dextrose on the Growth of S. cytophaga *in the Presence of Cellulose.*

Growth after	Concentration of solution (per cent.)								
	Control	0·005	0·01	0·025	0·050	0·10	0·25	0·50	1·00
3 days	+ +	+ +	– +	– +	–	–	–	–	–
8 days	+ + +	+ + +	+ + +	– +	– +	–	–	–	–
12 days	+ + +	+ + +	+ + +	+ + +	+ +	–	–	–	–

A further series was designed to show the difference in inhibitory power of two bioses, viz. saccharose and maltose, in the presence of cellulose. In the following table the records refer to actual growth but not to intensity of growth as in the other series.

TABLE VII.

Relative Inhibition of Growth by Saccharose and Maltose in the Presence of Cellulose.

		Concentration of solution (per cent.)										
		0·004	0·009	0·018	0·037	0·075	0·15	0·31	0·62	1·25	2·50	10·00
Saccharose	4 days	+	+	+	+	+	+	+	–	–	–	–
	6 ,,	+	+	+	+	+	+	+	+	+	–	–
	9 ,,	+	+	+	+	+	+	+	+	+	–	–
Maltose	4 days	–	–	–	–	–		–	–	–	–	–
	6 ,,	+	+	–	–	–	–	–	–	–	–	–
	9 ,,	+	+	+	–	–	–	–	–	–	–	–

The striking difference in effect of a reducing and a non-reducing sugar is well brought out, the critical concentration of maltose being in the region of 0·018 per cent., and of saccharose approximately 1·25 per cent., i.e. a ratio of 1 : 70. So far as we are aware, this specific susceptibility is quite unique.

Having determined the behaviour of the organism towards representatives of the carbohydrates, it was decided to proceed to an examination of the value of a number of organic acids as sources of carbon.

The general plan was similar to that adopted in the case of the carbohydrate series, i.e. cultivations were attempted in the absence and presence of cellulose. As we were in possession of a number of calcium salts of certain mono- and di-basic acids it was most convenient to carry out the tests with these. Unfortunately, the use of calcium salts introduces a serious complicating factor in that, as the solubility

of certain of the salts is very low, they tend to show only slight inhibitory powers.

TABLE VIII.

Relative Effects of Organic Calcium Salts.

Growth after 9 days	Concentration	Calcium formate	Calcium acetate	Calcium propionate	Calcium butyrate	Calcium oxalate	Calcium malate	Calcium tartrate	Calcium citrate
Without cellulose	0·1 %	–	–	–	–	–	–	–	–
	1·0 %	–	–	–	–	–	–	–	–
With cellulose	0·1 %	+	+	–	–	+ +	+ +	+ +	+ +
	1·0 %	–	–	–	–	–	–	+	+ +

From these experiments the conclusion appears justified, therefore, that representatives of the lower fatty acids, and of di- and tri-carboxylic acids, are equally unsuitable as sources of carbon as are the carbohydrates, since the organism completely failed to grow in any of the solutions in the absence of cellulose. In regard to their inhibitory power, both calcium formate and acetate are only slightly inimical in 0·1 per cent. solutions and this also appears to be the case with malate, oxalate, tartrate and citrate. This might at first be explained on the basis that the maximum solubility of the latter compounds is lower than the amount of the salt actually added, but this assumption makes it difficult to account for the obvious inhibition in the 1·0 per cent. solutions. It should be remembered, however, that the possibility of a slight amount of interaction with the constituents of the mineral salt solution and the formation of more soluble alkali salts is not entirely excluded. Both calcium propionate and calcium butyrate show inhibitory effects in 0·1 and 1·0 per cent. solutions.

The above work on the nutrition of the organism may be summarised as follows. The nitrogen requirements of *S. cytophaga* may be fulfilled by a range of nitrogen compounds—ammonium salts, nitrates, amides, amino-acids and peptone—provided that these, and especially the organic compounds, are not present in other than low concentrations. With peptone this is particularly the case, the critical concentration having been found to lie in the region of 0·25 per cent. Ammoniacal and nitric nitrogen are, on the whole, markedly superior to the other forms tested and may be supplied in much higher concentrations without inhibitory effects being introduced. *S. cytophaga* appears to be specific in its relation to carbon sources and its requirements are only met by cellulose. Not only are other compounds—higher alcohols, organic acids

and various carbohydrates—unsuitable for nutrition of the organism, but some, namely those compounds possessing marked reducing properties, have been found to be toxic even in very low concentrations, e.g. 0·018 per cent. maltose or 0·050 per cent. dextrose.

As regards the distribution and action of the organism in nature, it might be thought that, by virtue of its sensitiveness to cultural conditions, *S. cytophaga* would play a somewhat subordinate rôle. It is not improbable, however, that the conditions obtaining in the soil—aeration, presence of ammoniacal and nitric compounds and of plant residues, as well as the absence of appreciable quantities of soluble organic compounds—constitute ideal conditions for the growth of the organism.

In this relation to soil biological changes it may be of interest to set up a comparison, between *S. cytophaga* and the two other soil forms possessing monotropic relations to carbon compounds—the nitrous and nitric organisms. Such a comparison is made in Table IX, the data respecting the nitrifying bacteria being obtained from Winogradsky and Omelianski's paper[1].

TABLE IX.

Comparison of the Behaviour of Nitrosomonas, Nitrobacter and Spirochaeta cytophaga *towards Soluble Organic Compounds.*

	Inhibitory concentrations (per cent.)				
	Nitrosomonas		Nitrobactor		*S. cytophaga*
	min.	max.	min.	max.	max.
Sodium acetate	0·5	>1·5	1·5	3·0	>0·10*
Sodium butyrate	0·5	>1·5	0·5	1·0	>0·10*
Asparagin	0·05	0·3	0·05	>1·0	>0·50
Carbamide	>0·20	?	0·50	>1·0	>0·50
Peptone	0·025	0·20	0·8	1·25	0·25
Dextrose	0·025	0·20	0·05	0·2–0·3	0·05
Maltose	?		?		>0·018
Saccharose	?		?		>1·250

* Calcium salt used.

S. cytophaga is considerably more sensitive than either of the nitrifying bacteria, practically the sole exception being the high sensitiveness of Nitrosomonas to asparagin. The three organisms have certain common features such as their sensitiveness towards dextrose and their greater intolerance of butyrates than of acetates. It is somewhat remarkable that these three soil forms, two of which obtain their carbon as carbon

[1] Winogradsky, S. and Omelianski, W., *Cent. Bakt. Par.* II. 1899, 5, 436.

dioxide and the other which appears to rely on the most resistant carbohydrate, should exhibit this pronounced intolerance of soluble organic compounds.

General Aspects of the Cultivation of *S. cytophaga*.

Mention has already been made of some early attempts to secure elimination either of the thread or of the sporoid form of the organism by means of differential treatment. Although the object of the experiment was not achieved, the data thus obtained possess a few points of cultural interest. They relate to the effect of (*a*) reaction of the medium, (*b*) temperature, and (*c*) disinfectants.

Relations to reaction of the medium. Two series of experiments have been carried out, and the results secured indicate a fairly wide range of resistance to acid and alkaline conditions. The gradations in the reactions of the two sets are not identical and this is reflected in a certain amount of divergence in the results. In the first series there appears to have been reluctance to grow in concentrations exceeding N/80 acid and N/160 alkali; in the second series a tolerance was found ranging from N/50 acid to N/55 soda or, expressed in the terms in common bacteriological use, from $-2°$ to $+2°$. In view of the fact that liquid, and not solid, medium was employed, this range of resistance is somewhat noteworthy.

Table X.

Influence of the Reaction of the Medium on Growth.

Reaction of the culture solution											
HCl						NaOH					
N/20	N/40	N/80	N/160	N/320	N/640	N/640	N/320	N/160	N/80	N/40	N/20
–	–	+	+ +	+ +	+ +	+ +	+ +	+ +	–	–	–
N/25	N/50	N/100	N/130	N/300	N/720	N/800	N/160	N/100		N/55	N/25
–	– +	+ +	+ +	+ + +	+ + +	+ + +	+ + +	+ +		– +	–

An approximately neutral reaction of the nutrient solution may, of course, be secured by the addition of calcium or magnesium carbonate, between the respective values of which there is little difference, but general experience shows that in the presence of these compounds the breakdown of the cellulose is less *apparent* than when the reaction of the medium is due to alkaline carbonates.

Relations to temperature. The inquiries under this head are divisible into two distinct lines, viz. the determination of (1) the optimum

temperature for cellulose decomposition, and (2) the thermal deathpoint of the organism.

In pursuance of the first of these objects six portions of 2 grams each of pure cellulose fibre were placed in flat bottomed cultivation vessels and to each was added 100 c.c. of mineral salt solution with 0·25 per cent. sodium nitrate, the solution having been rendered slightly alkaline to phenolphthalein. The flasks were then inoculated with 1 c.c. of a culture of the organism and incubated at 20°, 25° and 30° for 14 days. At the end of this period the resulting cultures were acidified slightly, filtered, and the residues dried and weighed. The losses of cellulose at the different temperatures were found to be as follows:

20° (*a*)	Cellulose decomposed	0·334 grm.
20° (*b*)	,, ,,	0·302 grm., mean 0·318 grm.
25° (*a*)	,, ,,	0·370 grm.
25° (*b*)	,, ,,	0·414 grm., mean 0·393 grm.
30° (*a*)	,, ,,	0·414 grm.
30° (*b*)	,, ,,	0·446 grm., mean 0·430 grm.

The most vigorous decomposition thus took place in the region of 30°. Owing to limited incubator capacity at that time it was not possible to carry out a more extensive series, but subsequent experience has shown that although decomposition does proceed at 35°, this is not so rapid as at 30°. Moreover, the organisms exhibit certain abnormal features and have also been found to display a marked reluctance to grow in high dilutions at the higher temperature.

The determination of the thermal deathpoint has been carried out on a number of occasions, and the results of two series are given.

Series I. Length of Exposure—5 minutes.

Growth after	48°	50°	52°	56°	58°	60°	62°	62·5°	66°
6 days	+ +	+ +	+ +	+ +	+ +	+	–	–	–
15 days	+ +	+ +	+ +	+ +	+ +	+ +	+ +	–	–

Series II. Length of Exposure—10 minutes.

Growth after	40°	42°	44°	46°	48°	50°	52°	54°	56°	58°	60°	62°
7 days	+	+	+	+	+	+	+	+	+	–	–	–
9 days	+	+	+	+	+	+	+	+	+	+	–	–

In the two series the organism succumbed after exposure to a temperature of 62° for 5 minutes or a temperature of 58° for 10 minutes. In its thermal deathpoint *S. cytophaga* thus resembles the majority of the non-sporogenous organisms, as compared with the sporogenous

bacteria whose deathpoint is generally very much higher. It is, therefore, probably safe to assume that the formation of the sporoid stage does not confer on the organism any increased resistance to high temperatures.

Behaviour towards disinfectants. The susceptibility of *S. cytophaga* to volatile antiseptics was noted in some of the early experiments. Treatment of crude cultures with toluene or toluene vapour invariably resulted in the destruction of *S. cytophaga* and the persistence of contaminating sporogenous forms which, of themselves, were incapable of inducing typical decomposition of cellulose. Hence, it might be inferred that the power of resistance to disinfectants would be similarly low, and this is brought out by experiments with phenol. The tests were carried out in sodium ammonium phosphate-mineral salt solution with cellulose.

TABLE XI.

Effect of Phenol on S. cytophaga.

	Concentration of phenol (per cent.)							
Growth after	0·004	0·008	0·017	0·035	0·070	0·15	0·31	1·25
5 days	+	– +	–	–	–	–	–	–
9 days	+ +	+	–	–	–	–	–	

Microscopical examination of the culture with 0·008 per cent. of phenol showed the presence of both the thread and sporoid forms and a decided tendency to the production of highly granular filaments. The organisms were evidently already suffering from the effects of the phenol without, however, either of the forms being entirely suppressed.

Destruction of cellulose, etc. Under favourable conditions as to nitrogen and oxygen supply, the decomposition of filter paper, cotton wool, parchment paper and gun cotton proceeds with fair rapidity and, in fact, limitation of the breakdown changes frequently arises solely through exhaustion of the nitrogen supply of the medium. In this connection the results of two experiments may be given. In the first experiment a culture was made in a 2000 c.c. Erlenmeyer flask containing 200 c.c. of normal solution, i.e. with 2·0 grms. sodium ammonium phosphate per litre, together with 5·0 grms. of filter paper, the absolute dry weight of which was 4·66 grms. After eight days at 25° the culture was boiled, filtered through a tared paper, washed with hot water, dried and weighed. The weight of this residue was found to be 4·28 grms. thus indicating a loss of 0·38 grm. of cellulose in eight days. Taking into consideration the fact that there is generally no apparent growth

of *S. cytophaga* in less than three to four days after inoculation, the above loss of cellulose is by no means inconsiderable.

A second experiment shows the relation between cellulose breakdown and nitrogen supply. A number of flasks were prepared each with 2·0 grms. air-dry cotton wool and 100 c.c. of neutral nitrogen-free mineral salt solution. After sterilisation, four of the flasks received a sterile solution of sodium ammonium phosphate so that the concentration of the latter in the different culture flasks was equal to 0·5, 1·0, 2·0 and 4·0 grms. of the salt per litre of culture solution. After inoculation and incubation for 21 days at 25° the culture in each flask was treated with dilute (3·3 per cent.) hydrochloric acid, boiled, filtered, and the residue washed with distilled water and dried. The various data are given in Table XII.

TABLE XII.

The Relation of Nitrogen Supply to Cellulose Decomposition.

Nitrogen supplied	Wt. of cotton wool (dried at 100°) after 21 days	Loss	Ratio of nitrogen supplied: cellulose decomposed
nil	1·810 grms.	nil	—
3·51 mgrms.	1·712 „	0·098 grms.	1 : 27·5
7·14 „	1·580 „	0·230 „	1 : 32·2
14·28 „	1·415 „	0·395 „	1 : 27·7
28·56 „	1·460 „	0·350 „	1 : 12·3

The ratios for the three lower concentrations of nitrogen show good agreement among themselves, the amount of cellulose destroyed being about 30 times the quantity of nitrogen originally present. This consistency may be taken as indicating that the whole of the nitrogen had been utilised. With the most concentrated solution, the rapidity of the action appears to have been checked; it is also possible that the whole of the nitrogen has not been completely utilised, but unfortunately an actual determination of the residual nitrogen was not made.

By-products of growth of S. cytophaga. Active cultures of *S. cytophaga* have three main characteristics which may be stated to be (*a*) pigment production, (*b*) the absence of obvious gas formation and (*c*) resolution of the cellulose into a glistening mucilaginous mass.

Pigment production. Growth of the organism on filter paper or cotton wool, and especially when the reaction of the medium is slightly alkaline, gives rise to the formation of a brilliant yellow pigment. It may be extracted with ease from the liquid or dried cultures by means of alcohol, the extract giving on concentration a dark yellow or orange coloured oily residue. The pigment goes into solution with the ordinary

fat solvents, petroleum ether, benzol, chloroform, carbon bisulphide, ether, etc. The first three yield a bright yellow solution, whilst that with carbon bisulphide is orange yellow, and that with ether canary yellow, in colour. This colour is intensified by alkali and destroyed by weak mineral acids.

The pigment gives reactions approaching those of the carotin group; the production of a blue colouration on exposure to the action of concentrated sulphuric acid is somewhat feeble, but strong hydrochloric acid gives a deep dirty green colour. Hence it resembles the lipochrome substances which are formed by many of the bacteria such, for example, as *Sarcina lutea* and *aurantiaca* and *Staphylococcus pyogenes aureus*. Up to the present we are unable to adduce any evidence as to the physiological importance of the pigment.

Acid products. During the decomposition of cellulose the reaction of the medium undergoes a gradual change so that after 8–10 days the liquid is distinctly acid to litmus. This is not due, as might be supposed, to the abstraction of the ammonium radicle from the phosphates supplied, and the formation of acid phosphates, since the same change proceeds in culture solutions with sodium nitrate. On the other hand, there are indications of the production of small quantities of volatile fatty acids. Old cultures of the organism, when heated with concentrated sulphuric acid, give rise to a distinct smell of butyric acid, and by the addition of ethyl alcohol a smell resembling that of ethyl butyrate is evident. In this connection two cultures were prepared, each with 100 c.c. of mineral salt solution containing 0·287 grm. of precipitated cellulose, in one case with, and in the other without, calcium carbonate. At the end of eight days each culture was steam-distilled and gave a distillate slightly acid to litmus. On titration 2·5 and 3·1 c.c. of N/10 sodium hydroxide solution were required by the respective distillates; this acidity if entirely due to butyric acid would be equal to approximately 7–9 per cent. of the original cellulose. By analogy with other carbohydrate-fatty acid fermentations, it is not improbable that other fatty acids are produced, but their identification and estimation lies outside the scope of the present inquiry.

Mucilage. By the continued growth of *S. cytophaga* for a few days, cellulose material at, or slightly above, the level of the culture solution becomes distinctly mucilaginous and this property is also gradually acquired by the culture solution. The latter is difficult to filter when cold, but presents no difficulties when brought to the boil or after being slightly acidified. It was originally supposed that this mucilaginous

substance might be of a dextrine nature and possibly the precursor of saccharine compounds. Examination of numerous cultures of different ages has failed, however, to indicate the presence of any breakdown product capable of reducing Fehling's solution or possessing any optical activity. The non-dextrin character of the mucilage is shown by the results of an experiment in which a quantity of the substance was exposed to the action of Taka-diastase. After treatment according to the routine method the mucilage solution "failed to show even the slightest trace either of sugar or dextrin." A further attempt was made to obtain hydrolysis of the mucilage by means of fuming hydrochloric acid. Under these conditions cellulose gives rise to the production of dextrose; after digestion for three hours with hydrochloric acid, mucilage solution was utterly lacking in optical activity[1]. Hence a fundamental difference exists between the nature of the mucilage and of cellulose. On the other hand, the mucilage possesses certain points of resemblance to the pectin or pectic acid group of compounds. On various occasions, cultures of the organism have been reduced to dryness *in vacuo* and extracted in alcohol to remove the pigment. On treatment of the residue with cold water a slight, and with hot water a still greater, amount of mucilage was brought into solution. After a partial concentration of the extract the mucilage was thrown down with alcohol and filtered. The residue, when treated with cold water, yielded a thick viscid fluid which was extremely difficult to filter. On being heated, but without being brought to the boil, the solution could be filtered with ease.

It is, of course, probable that such a solution contains a whole range of degradation products. Tannic acid fails to effect any precipitation. On the addition of hydrochloric acid the solution is converted into a thick jelly which on shaking and being allowed to stand, or on heating, assumes the form of a light semi-transparent membranous precipitate. Precipitation also results from the addition of barium and calcium hydroxide, basic and neutral lead acetate, barium and calcium chloride, silver nitrate and magnesium sulphate.

The solution prepared in the above manner is neutral to litmus. Treatment of the mucilage with hot dilute hydrochloric acid gives a precipitate which is insoluble in water, but is readily soluble in ammonia, from which it may again be thrown down on acidification. We thus have a number of reactions which are also exhibited by pectin derivatives,

[1] We desire to express our indebtedness to Mr W. A. Davis and Mr E. Horton, of this Laboratory, for kindly carrying out the Taka-diastase and acid conversion tests respectively.

but further work is required before the chemical character of the mucilage can be definitely established. As far as soil conditions are concerned, it is not improbable that an extensive production of mucilage from plant residues would exert some action on the physical behaviour of the soil; from the chemical standpoint, and on account of its insolubility in acids and solubility in ammonia, the mucilage would, without doubt, appear in the "crude humus" fraction in the conventional soil analysis.

The Relation of Cellulose-decomposing Organisms to Nitrogen Fixation.

Since this, and a preceding investigation on nitrogen fixation, arose from the necessity of accounting for the observed gains in nitrogen in soils that had been allowed to revert to prairie conditions, we may be permitted to refer briefly to the relation between cellulose, as distinct from crude plant residues, and the assimilation of atmospheric nitrogen. In the first instance it was supposed that although cellulose is not directly available to such nitrogen-fixing organisms as Azotobacter, the products of decomposition would include sugars such as are formed on the hydrolysis of cellulose by acids, and that these compounds would serve as sources of energy for Azotobacter. It has already been seen, however, that the formation of sugars during decomposition is highly problematical, but that there are indications of the formation of volatile fatty acids, and the value of some of these for nitrogen-fixation has frequently been observed[1]. Moreover, there is definite evidence that the cellulose breakdown products do increase nitrogen-fixation and in this connection the following results are submitted.

Two sets, each of six flat bottomed flasks, were prepared; one set (A) received 50 c.c. of mineral salt solution containing 0·1 per cent. mannite and, in addition, 1·0 grm. each of cellulose and calcium carbonate. The second set (B) received the same additions with the exception that in this case the mannite was replaced by a supply of sodium nitrate equal to 0·010 per cent. nitrogen, the main object of this variation being to ascertain whether the associative action could be initiated equally well by nitrogen-fixation (set A) or cellulose decomposition (set B). From the results given below it will be seen that this was not the case and set B actually lost nitrogen—possibly by the action of such denitrifying cellulose-decomposing bacteria as were observed by van Iterson[2].

[1] Mockeridge, F. A., *Biochem. J.* 1915, **9**, 272–283.
[2] Iterson, C. van, *loc. cit.* 690.

TABLE XIII.

Associative Growth of Nitrogen-fixing and Cellulose-decomposing Organisms.

	Mean total N after 4 weeks		
	Control	Azotobacter alone	Azotobacter and crude culture of cellulose organism
Set A (mannite)	0·735 mgrms.	0·91 mgrms.	2·835 mgrms.
Set B (sod. nitrate)	7·17 „	6·44 „	4·69 „

The presence of 0·05 grm. of mannite failed to give any increase of nitrogen over the control, but the addition of 1·0 grm. of cellulose together with cellulose-decomposing organisms gave what may be regarded as a definite increase.

A similar experiment was also carried out, but in this case the solution contained 1·0 per cent. mannite; 50 c.c. solution and 1·0 grm. cellulose were again taken for each flask. The results are:

	Total nitrogen in flasks after 3 weeks		
	Control	Azotobacter alone	Azotobacter and cellulose organisms
(*a*)	1·12 mgrms.	4·20 mgrms.	5·81 mgrms.
(*b*)	1·82 „	3·01 „	7·42 „

The results thus show a fixation of 4·27 mgrms. nitrogen per gram of mannite, and a supplementary fixation of 3·01 mgrms. when cellulose was also supplied.

In a third experiment the amount of cellulose lost during the fermentation was ascertained prior to the determination of total nitrogen. The mean nitrogen content of the respective flasks was found to be 1·19, 2·78, and 5·74 mgrms.: the loss of cellulose during fermentation amounted to 0·153 grm. From these data it may be calculated that the fixation of nitrogen per gram of mannite *supplied* was equal to 3·18 mgrms., while that per gram of cellulose *actually decomposed* was no less than 19·3 mgrms. All three experiments provide definite indications of the value of cellulose breakdown products for the assimilation of atmospheric nitrogen. Somewhat analogous results have also been obtained by the combined growth of anaerobic cellulose-decomposing and anaerobic nitrogen-fixing bacteria[1].

[1] Pringsheim, H., *Cent. Bakt. Par.* II, 1909, **23**, 300–304; 1910, **26**, 222–227.

Summary.

From the foregoing account the following summary may be given:

1. Examination of Rothamsted soils on different occasions has revealed the presence of an organism capable of breaking down cellulose with comparative ease.

2. This organism presents a number of features of morphological and physiological interest. Morphologically, the organism appears to possess greater affinities with the Spirochaetoideae than with the bacteria and the name *Spirochaeta cytophaga* is, therefore, suggested.

3. While the spirochaet is capable of considerable vegetative growth as a sinuous filamentous cell, it also appears to pass through a number of phases which terminate in the production of a spherical body (sporoid) which differs in a number of respects from the true spores of the bacteria. Germination of the sporoid again gives rise to the filamentous form, which possesses perfect flexibility and is feebly motile. The latter does not apparently possess flagella.

4. *Spirochaeta cytophaga* is essentially aerobic; its optimum temperature is in the region of 30°. Both the thread and sporoid stages are killed by exposure to a temperature of 60° for ten minutes.

5. The nitrogen requirements of the organism may be met by a number of the simpler nitrogen compounds—ammonium salts, nitrates, amides and amino-acids. Peptone is also suitable in concentrations up to 0·025 per cent. Stronger solutions, e.g. 0·25 per cent., lead to marked inhibition of growth. The organism fails to grow on the conventional nutrient gelatine or agar.

6. Comparative experiments with a number of higher alcohols, sugars and salts of organic acids show that none of these is capable of meeting the carbon requirements of the organism. Cellulose is the only carbon compound with which growth has been secured.

7. Although none of the monoses, bioses and other carbohydrates tested is able to support growth, many of them exert an inhibitive action on cellulose decomposition if present in other than very low concentrations. This may be correlated with the reducing properties of the carbohydrate. Maltose, for example, has been found to be approximately 70 times more toxic than saccharose.

8. Of the various by-products of the action of *Spirochaeta cytophaga* may be mentioned (*a*) a pigment possessing relations to the carotin

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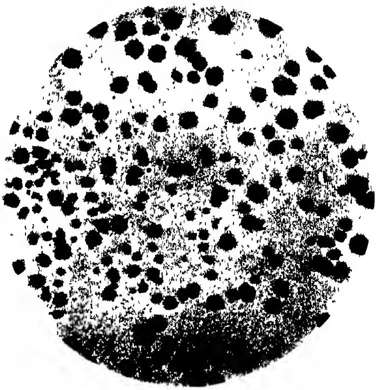

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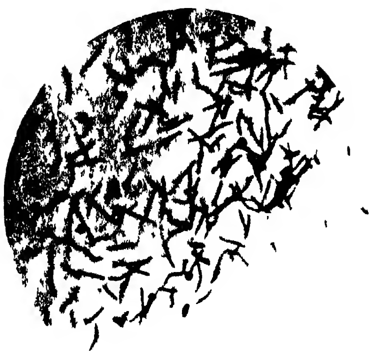

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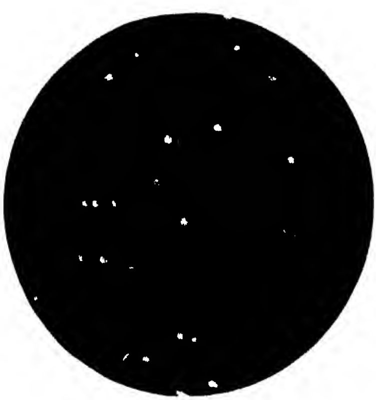

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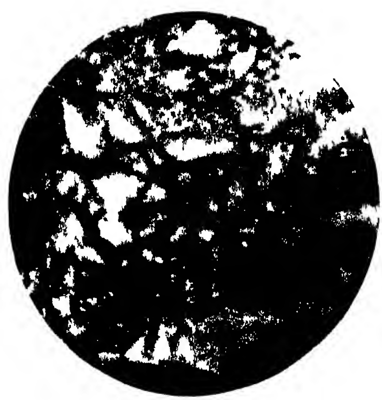

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group, (*b*) mucilage which does not give rise to optically active compounds on hydrolysis and (*c*) small quantities of volatile acids.

9. Evidence is also adduced to show the relation of cellulose decomposition to the assimilation of atmospheric nitrogen.

DESCRIPTION OF ILLUSTRATIONS ON PLATES I–III.

(Photomicrographs—magnification 1000 diameters.)

PLATE I.

Fig. 1. Test tube cultures with mineral salt solution and filter paper; 4, 7, and 12 days old.

Fig. 2. Petri dish culture on sodium nitrate-mineral salt agar with filter paper superimposed. Showing holes in paper by the growth of *S. cytophaga* (nat. size).

Fig. 3. Photomicrograph of young culture of *S. cytophaga* in filter paper tube. Showing typical incurvation of thread form.

Fig. 4. Photomicrograph of young culture of *S. cytophaga* in liquid culture with precipitated cellulose. Showing well-marked sinuous forms.

Fig. 5. Photomicrograph of *S. cytophaga* in *mass culture* on nutrient agar. Enfeebled forms exhibiting initial granulation of cells.

Fig. 6. Photomicrograph of young culture showing equatorial or polar segregation of chromatin substance. "Deposition" stain with alcoholic fuchsin.

PLATE II.

Fig. 1. Photomicrograph of culture on oat plant residue showing cells with chromatin "bridge" (below centre) and also sporoids.

Fig 2. Photomicrograph of culture on filter paper, incubated at 35°. All stages from the thread to the final sporoid are represented.

Fig. 3. Photomicrograph of same culture at a later stage, showing formation of a pre-sporoid stage with double granules (or band).

Fig. 4. Photomicrograph of culture showing (below centre) cells with (*a*) band, (*b*) bridge, and (*c*) double granules of chromatin. Also adjacent cell with internal coccoid structure.

Fig. 5. Photomicrograph of sporoids of different ages and, below, several apparently devoid of chromatin substance.

Fig. 6. Photomicrograph of older culture showing predominance of sporoids.

PLATE III.

Fig. 1. Final stage in the dissolution of a cellulose fibre.

Figs. 2, 3, and 4. Photomicrographs showing emergence of thread form from the sporoid. Preparation for Fig. 3 stained with alcoholic fuchsin.

Fig. 5. Photomicrograph of Indian ink preparation with sporoid and thread form.

Fig. 6. Photomicrograph of culture shown on Plate II, figs. 2 and 3, second generation after transference from 35° to 25°. Showing intense granulation of cells.

(*Received November 30th*, 1918.)

MEAT PRODUCTION.

By J. ALAN MURRAY, B.Sc.

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For purposes of discussion of the problems of meat production the term "condition" may be defined as the ratio of the amount of fat to the amount of non-fatty matter in the body of the living animal, and the term "fattening" as any alteration in this ratio. Such alteration may result from (*a*) increase, (*b*) decrease in the amount of fat, (*c*) increase, (*d*) decrease in the amount of non-fatty matter. This, of course, implies that fattening may be either positive or negative and that the "increase" may be mathematically a minus quantity.

The composition of the non-fatty matter is practically constant. It is the same in cattle, sheep and pigs and is not affected by condition but varies slightly with age. The averages, as deduced from Lawes and Gilbert's analyses, are as follows:

	Ash %	Protein %	Water %
Young animals (calves, lambs and pigs)	3·75	19·35	76·9
Adults (cattle and sheep)	5·60	21·84	72·6

The composition of the whole body is therefore determined by the amount of fat in it, and the relation between condition and live weight is expressed by $M = m\,(p - f)/(p - F)$, where m and M are the live weights corresponding to f and F per cent. of body fat respectively and p is the percentage of fat in the increase. When the amount of non-fatty matter remains unaltered—in some cases this can be inferred—$p = 100$.

As it is impossible to distinguish between fat formed in the course of growth and that due to fattening the total amount of fat in the body ($mf/100$) at any moment must be treated as fattening increase and the non-fatty matter $m\,(1 - f/100)$ as growth increase. The latter, it will be seen, is the live weight in fat-free condition.

For each pound of fat in the increase the animal must consume and resorb at least 1 lb. of fat or equivalent quantity of other nutrients. According to the theory of isodynamic replacement 2·3 lb. of starch is

equivalent to 1 lb. of fat. Digestible fat in the food does not, however, produce its own weight of body fat. When the increase consists entirely of fat:

$$N = 2{\cdot}3\,(M - m) \times 100/y,$$

where y is the percentage of metabolisable energy recovered in the gain and N is the amount of digestible nutrients (starch) required to produce the increase $(M - m)$.

When the increase consists entirely of non-fatty matter, of which about 22 per cent. is protein:

$$N - 22/100\,(M - m) \times 100/y,$$

where N is the amount of digestible nutrients (protein) required to produce the increase $(M - m)$.

It will be seen that the amount of food required to produce a pound of fat is more than ten times as much as is required for an equal increment of non-fatty matter. These however are particular and extreme cases, though not perhaps of infrequent occurrence in practice. Commonly the increase comprises both fat and non-fatty matter, due to simultaneous growth and fattening, and it must be considered as a whole.

If m and M be the live weights and f and F the percentages of fat in them respectively the amount of fat in the increase is $(MF - mf)/100$ and the amount of non-fatty matter is $[M\,(100 - F) - m\,(100 - f)]/100$. The nutrients required to produce the same are:

$$\text{Starch} = \frac{2{\cdot}3\,(MF - mf)}{100} \times \frac{100}{y};$$

and $$\text{Protein} = \frac{22}{100}\left\{\frac{M\,(100 - F) - m\,(100 - f)}{100}\right\} \times \frac{100}{y}.$$

This formula is perfectly general; it covers both of the cases previously considered. Of course, if the nutritive ratio of the dietary so calculated is more than about 12 to 1—in some cases it may be much less—it must be reduced by substitution of protein for starch in equivalent quantity. On this understanding the formula may be simplified to:

$$N = 0{\cdot}23\left\{\frac{100\,(M - m) + 9\,(MF - mf)}{y}\right\},$$

where N is the total digestible nutrients (starch) required to produce the increase $(M - m)$.

N divided by T (duration of the fattening period) gives the average amount required per day. For example, if the data were, as in the

Rothamsted investigation, $m = 1131$, $M = 1334$, $f = 20{\cdot}81$, $F = 32{\cdot}02$, the total amount of digestible nutrients required to produce the increase (203 lb.) would be about 800 lb.; and if the duration of the fattening period were 100 days the average amount would be 8 lb.—say 7¼ lb. of starch and ¾ lb. of protein—per day in excess of maintenance requirements.

There is at present no known method by which the percentage of fat in the bodies of living animals can be exactly determined; and until such is forthcoming it can only be inferred from the "condition" as estimated by practical experts. This is admittedly crude and unsatisfactory but it was considered good enough for the estimation of the composition of "fattening increase" by Lawes and Gilbert and it is, in effect, for that purpose it is now required. They established the following relations:

Condition (Oxen)	Body fat %	Condition (Sheep)	Body fat %
Half fat	20·8	Half fat	25·8
Fat	32·0	Fat	37·9
		Very fat	48·3

Atwater and Bryant[1] found that the percentages of fat in sides of beef varied as follows:

Condition	Fat (%)
Very lean	from 0·7 to 4·7 average 2·7
Lean	„ 10·1 „ 11·7 „ 10·6
Medium fat	„ 12·7 „ 21·9 „ 18·1
Very fat	31·6

It appears therefore that the verbal terms used by practical experts to describe condition may be interpreted approximately as follows:

Condition	Body fat, %
Very lean	less than 5
Lean	from 5 to 10
Store	„ 10 „ 15
Fair (good store)	„ 15 „ 20
Half fat	„ 20 „ 25
Moderately fat	„ 25 „ 30
Fat	„ 30 „ 35
Prime	„ 35 „ 40
Very fat	„ 40 „ —

The value of y is tolerably well known. Kellner[2] found that ruminants produce from 474 to 598 grams of body fat from 1 kg. of digestible fat

[1] *Bull.* 28, U.S. Dept. of Agric., "Chemical composition of American Food Materials."
[2] *Scientific Feeding of Animals*, p. 82.

in the food when the pure nutrient is added to an adequate basal ration. In a recent experiment Armsby[1] found that 57·3 per cent. of the metabolisable energy in excess of maintenance requirements was recovered in the gain made by an unfattened steer and 56·4 per cent. in that made by the same animal in fat condition. Moulton[2], quoted by Armsby, "computed that in a fat and a very fat steer 53·39 per cent. and 52·49 per cent. respectively of the metabolisable energy supplied in excess of maintenance was recovered in the gain." It appears therefore that the value of y in fattening foods is between 50 and 60 and that, apart from the variation in individuals, it is practically a constant quantity. At all events it is not materially affected by the condition of the animal.

It is well known, however, that the return for food consumed diminishes rapidly as condition improves. In the same experiment Armsby found that 5·2 lb. of digestible organic matter was consumed for each pound of increase made by the unfattened animal and 9·6 lb. —nearly twice as much—after fattening. He attributes this to increased requirements for maintenance.

This conclusion might have been anticipated—it was in fact anticipated by the author—from the fact that, when the rations of a fat beast are reduced to what is adequate for maintenance in store condition, it "goes back," i.e. it loses weight and becomes lean again. It is evident also that, in the case of fully grown animals, the return for food consumed must eventually fall to zero. In other words there must be a point, somewhere, beyond which an animal cannot be further fattened, the maximum amount of food it can consume being just sufficient to maintain it in that condition.

This point has not been exactly determined but available data serve to narrow the field of enquiry. If condition be expressed in terms of percentage of body fat the mathematical limit is 100. The actual limit must be something short of that for it is physiologically impossible for the body to consist entirely of fat. On the other hand it cannot be less than 48·3 per cent. for that amount of body fat has been found in a "very fat" sheep. Other considerations indicate that the limit of fatness lies between 50 and 60 per cent. of body fat. The point is probably not well defined as it depends mainly upon the animal's capacity for food. The specific capacity for food—the ratio of dry matter consumed (when given food *ad lib.*) × 1000 to the fat-free live weight—was, in the case of this particular animal, [9·9116 × 1000/410·4 =] 24·22.

[1] *Journ. Agr. Research*, **11**, No. 10.
[2] *Journ. Biol. Chem.* **31**, No. 2.

In Armsby's experiment the animal was a three-year-old steer; and it may be inferred from the description of its condition that it contained about 20 per cent. of body fat before and 35 per cent. after fattening. Accordingly the "fattening increase" must have comprised about 80 per cent. of fat and 20 per cent. of non-fatty matter. This indicates that the animal had grown a little whilst fattening.

The observed basal katabolism was 5563 Cal. and 7544 Cal. in the two conditions respectively. In order to ascertain the influence of condition, as apart from growth, the latter number must be reduced in proportion to the two-thirds power of the fat-free live weight. Taking for this purpose the mean weights (513 kg. before and 648 kg. after fattening) the corrected figures, i.e. for animals of the same size, are as follows:

	Unfattened	Fattened
Condition (body fat, %)	20	35
Live weight (kg.)	513	631
Basal katabolism (Cal.)	5563	7410

It will be seen that basal katabolism increases faster than live weight; but it is fairly obvious that the greater weight to be supported is, to a large extent, the cause of the increase. It is to be expected, therefore, that the curve of basal katabolism will be similar in form to the curve of live weight but will rise more steeply. The latter is expressed by $M = 100m\,(100 - f)$, where m is the fat-free live weight and f the percentage of body fat. If basal katabolism increased in the same proportion as the live weight the curve would be $B = 100b/(100 - f)$, but as it rises more steeply the formula must be

$$B = \frac{100b}{100 - af},$$

where a is an empirical number, b is the basal katabolism in fat-free condition and B is basal katabolism in the same animal when it contains f per cent. of body fat.

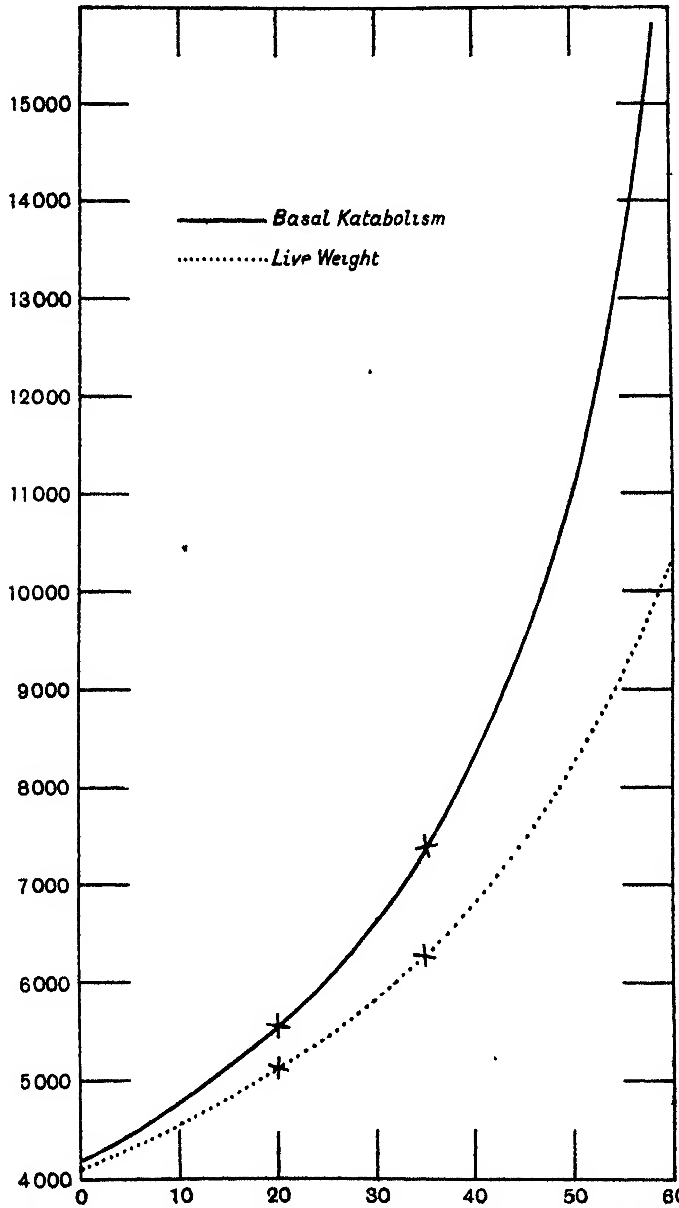
From the data above both a and b can be determined, thus—

$$\frac{100b}{100 - 20a} = 5563 \quad \text{and} \quad \frac{100b}{100 - 35a} = 7410,$$

$$\therefore\ a = 1{\cdot}247 \text{ and } b = 4175{\cdot}59.$$

The live weights and basal katabolism corresponding to different percentages of body fat are given in the table below and the curves shown in the diagram were plotted from the same. The points marked × are those determined in the experiment.

Condition (body fat, %)	0	10	20	30	40	50	60
Live weight (kg.)	410	456	513	586	684	821	1026
Basal katabolism (Cal.)	4175	4770	5563	6671	8331	11090	16580



Basal katabolism (Cal.) and live weight (hectograms) on OY and body fat (per cent.) on OX.

The striking divergence towards the upper ends of the curves may possibly cause the method to be regarded with suspicion. It is, however, precisely this circumstance that tends to confirm it. When the animal was given unlimited food the quantity consumed was 9·9116 kg. of dry matter = 27865 Cal. of metabolisable energy. Of this amount it is computed that [9·9116 × 1256 =] 12449 Cal. was expended on the food ingested. When this is deducted and correction for size is made there remains 15140 Cal. all of which would be required for maintenance in the condition of maximum fatness. The curve indicates that this is 58 per cent. of body fat, a number which agrees tolerably with the forecast previously made.

In view of the paucity of the experimental data and the conjectural character of the estimates of body fat no great confidence can be placed in these conclusions. It is probable, however, that the diagram represents the general trend of the curve of basal katabolism and, within limits of practical importance—say from 10 to 40 per cent. of body fat —it may be regarded as a close approximation to the truth for this particular animal.

It has been demonstrated that basal katabolism does not vary as the live weight or as the two-thirds power of the same when the variation in the latter is wholly or mainly due to alteration of condition; that it varies approximately as the two-thirds power of the live weight for animals in like condition is generally accepted. The basal katabolism in fat-free condition, therefore, suggests itself as the natural fundamental unit for estimation of maintenance requirements. The calculation on this basis involves three distinct steps, viz. (1) reduction of observed live weight to fat-free condition, (2) calculation of basal katabolism in proportion to the two-thirds power of the same, (3) rectification of the result according to the observed condition. Assuming the accuracy of the data here considered, this is more concisely expressed by:

$$B = \frac{4175\cdot6}{410\cdot4^{\frac{2}{3}}} \times \left\{\frac{M(100-f)}{100}\right\}^{\frac{2}{3}} \times \frac{100}{100 - 1\cdot247f},$$

or $$\log B = \tfrac{2}{3}\log\left\{\frac{M(100-f)}{100}\right\} - \log(100 - 1\cdot247f) + 3\cdot8786,$$

where f is the percentage of body fat and B is the basal katabolism corresponding to M the observed live weight (kg.).

Results obtained by use of this formula are tabulated below for comparison with those calculated in proportion to the two-thirds power of the live weight.

Condition (body fat) (%)	Live weight (kg.)	Basal katabolism: By formula (Cal.)	Basal katabolism: In proportion to $\frac{2}{3}$ power of live wt. (Cal.)	Difference (Cal.)
0	410	4175	4794	−619
10	456	4770	5144	−374
20	513	5563	5563	—
30	586	6671	6080	591
40	684	8331	6741	1590
50	821	11090	7612	3378
60	1026	16580	8833	8247

The following table shows the basal katabolism calculated by the formula for animals in different condition but each having a live weight of 454 kg. (1000 lb.):

Condition (body fat, %)	0	10	20	30	40	50	60
Basal katabolism (Cal.)	4470	4761	5133	5619	6345	7533	9636

As between the lean (10 per cent.) and fat (40 per cent.) condition the difference amounts to 1584 Cal. It appears, therefore, that the maintenance requirements of two animals of the same live weight may differ by more than 30 per cent. The method of computing maintenance rations in proportion to the two-thirds power of the live weight without regard to condition has always been open to suspicion. In view of these figures it appears to be quite inadmissible.

(*Received September* 14*th*, 1918.)

NET ENERGY VALUES AND STARCH VALUES.

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It is now accepted as a fundamental doctrine in animal nutrition that the prime function of food is to supply energy for the operation of the human or animal body and that all its other diverse uses are essentially tributary to this main purpose. The growing recognition of this fact in its relations to the nutrition of farm animals has given rise during the past twenty-five years to extensive investigations, especially by Zuntz and his associates, by Kellner and Köhler and by the writers, in which the attempt has been made to determine experimentally how much energy the various feeding stuffs can actually contribute toward the upkeep of the animal body.

Zuntz's investigations have been chiefly upon the horse and will not be discussed here. Kellner's and our own have been upon cattle. For them the same general methods have, in the main, been used and the results have been in general accord. In the actual application of those results to practice, however, two quite diverse, although not fundamentally inconsistent, methods have been followed, with more or less resulting confusion. The matter has seemed to us to be of sufficient importance to warrant a brief contribution to the discussion of the subject and an attempt to make clear what we, at least, conceive to be the relation between the two systems.

The earlier of the two was Kellner's system of "Starch values," which before the war had obtained wide currency not only in Germany but in other countries of continental Europe and to some extent in Great Britain.

Kellner's starch values are in reality disguised energy values. In his fundamental investigations he used substantially the same general methods adopted subsequently by the writers; that is, he determined by so-called indirect calorimetry, using a Pettenkofer respiration apparatus, what part of the total energy contained in a variety of feeding stuffs could actually be recovered as gain of flesh and fat by the animal.

Furthermore, similar trials were made with nearly pure gluten, starch, cellulose, sugar and oil as representatives of the digestible protein, carbohydrates and fats of feeding stuffs, and from their results, corrected by those on actual feeding stuffs, a method was worked out for computing the energy values of materials not yet subjected to respiration experiments. The details of the method of calculation have been frequently published[1] and need not be reproduced here. The writers, following, as already stated, the same general plan of investigation but determining the actual heat production of the experimental animal as well as its respiratory products, and including experiments on submaintenance rations, have proposed[2] a somewhat simpler method of computation for attaining the same end.

Thus far, the two sets of values substantially parallel each other. It is in the method of expressing them for practical use in computing rations that the two systems diverge. The writers have expressed them directly in terms of energy ("Net energy values"), simply using a large unit (the *therm* = 1000 kilogram calories) to avoid the inconvenience of large numbers. Kellner, on the contrary, in view of the unfamiliar nature of energy units and of the large numbers required to express the energy values in terms of calories, was led to adopt as a substitute the equivalent amount of digested starch. Suppose, for example, that a sample of maize meal is found, either by direct experiment or by calculation, to have a net energy value of 85,200 calories per hundred pounds, or 85·2 therms, *i.e.* to be capable of contributing this amount to the upkeep of the body. According to Kellner's investigations, one pound of digestible starch has a net energy value of 1·071 therms. The 85·2 therms contained in one hundred pounds of maize meal might, then, be supplied by 85·2 ÷ 1·071 = 79·5 pounds of digestible starch and the latter number is the "Starch value," in Kellner's sense, of the maize meal. In other words, so far as the energy supply to the animal is concerned one hundred pounds of the maize meal might be replaced by 79·5 pounds of digestible starch, while conversely the starch value multiplied by 1·071 gives the net energy value in therms.

Kellner's starch value may be said to be a mixed unit. It attempts to express what are really quantities of energy in terms of matter and it has always seemed to us an unfortunate and an unnecessary concession to established usage. Moreover, experience has shown that in its actual

[1] Compare Kellner, *The Scientific Feeding of Farm Animals* (Goodwin's translation), and Armsby, *The Nutrition of Farm Animals*, pp. 668–672.

[2] *Journ. Agr. Research*, 3 (1905), 486; *The Nutrition of Farm Animals*, pp. 673–675.

use it is not always easy, even for experts, to avoid misapprehension and confusion of thought, as some striking instances in the recent literature of the subject have shown.

Misapprehensions regarding Starch Values.

Kellner's starch value in its proper sense as just defined has been mistakenly identified with two quite different conceptions.

The first of these is the so-called "Carbohydrate equivalent" of the digestible nutrients. By conventional methods we determine the amounts of digestible protein, carbohydrates and fat contained in a feeding stuff and by multiplying the fat by 2·25 or a similar factor and adding the other two we compute the carbohydrate equivalent of the digested substances, or the "Total nutrients." Thus for average American alfalfa hay as reported by Henry and Morrison[1] the computation is

Digestible protein	10·6	per cent.
,, carbohydrates	39·0	,,
,, fat 0·9 × 2·25 =	2·0	,,
	51·6	,,

Such a calculation, however, simply shows that the digested nutrients in one hundred pounds of the hay contain roughly the same amount of total energy as 51·6 pounds of carbohydrates, viz. about 97·9 therms. It shows nothing whatever regarding the losses of energy which occur during digestion and assimilation nor as to how much remains available to the animal. While in a sense it is a starch equivalent it is not Kellner's starch value. It shows what the hay contains but not what useful effect it can produce.

A second misapprehension identifies Kellner's starch value with what has been called the "Physiological heat value," or the "Fuel value," or what the writers have designated as the "Metabolizable energy."

It is well recognized that not all the energy contained in the digested nutrients is available to the body, but that a considerable proportion of it escapes unused in the metabolic products of the urine and in the combustible gases produced in the digestive tract. In the case of starch some 10 per cent. of its energy escapes in the methane excreted by cattle, while none of it is lost in the urine. One pound of digested starch, therefore, is capable of yielding in the form of heat in the body of a

[1] *Feeds and Feeding*, 15th ed., p. 660.

steer only 1·706 therms out of its total energy content of 1·897 therms. The mean of similar determinations shows that, after deducting the losses in urine and methane, the digestible matter of one hundred pounds of average alfalfa hay, out of its total energy content of 97·9 therms, may supply 80·8 therms of heat to the body. This is its metabolizable energy, or fuel value. But since one pound of starch can supply 1·706 therms, evidently the digestible matter of one hundred pounds of the alfalfa hay yields as much heat as $80{\cdot}8 \div 1{\cdot}706 = 47{\cdot}37$ pounds of starch. In other words, 47·37 pounds of digested starch, instead of 51·6 pounds, would be equivalent as body fuel to one hundred pounds of the hay.

This figure evidently approximates more nearly to expressing a physiological value than the previous one, but even this reduced value does not measure the actual contribution which the hay can make to the nutrition of the animal, since it fails to take account of the very considerable amount of energy expended in the mechanical and chemical processes incident to the consumption, digestion and assimilation of the feed. It was precisely the special merit of Kellner's researches that they covered this point. He measured the actual amount of energy utilized by the animal and demonstrated that it was notably less, not only than the carbohydrate equivalent of the digested nutrients but also less than the physiological heat value, or fuel value, or metabolizable energy of these nutrients.

Moreover, not only is the utilizable or "Net" energy of a feeding stuff less than its fuel value, or metabolizable energy, but the two are not even approximately proportional. In Kellner's investigations, as summarized by the senior author[1], the percentage of the metabolizable energy which was actually utilized ranged, in round numbers, from 18 per cent. in wheat straw to 66 per cent. in cotton seed meal while in our own experiments there was a similar range from 45 per cent. in maize stover to 61 per cent. in hominy feed. In other words, to supply as much metabolizable energy as 100 pounds of hominy feed would require 180 pounds of maize stover, but to supply as much net, or utilizable, energy as 100 pounds of hominy feed would require 243 pounds of maize stover.

Kellner's starch values were intended to express the net, or utilizable, energy of the feed and not its content of carbohydrates nor its fuel value. Thus the starch value of average alfalfa hay, computed by Kellner's method, is 34·5 pounds per hundred, equivalent to 36·9 therms of net

[1] *The Nutrition of Farm Animals*, p. 660.

energy or to 45·7 per cent. of the fuel value, while by the writers' method of computation the net energy would be still less, viz., 32·3 therms.

Maintenance and Production Values.

Perhaps the most prolific source of confusion in the discussion of starch values and energy values has been the fact that Kellner, in his earlier publications, assumed that the fuel values, or metabolizable energy, of feeding stuffs as determined by him represented their values for maintenance. This was in accord with the notion then current and still more or less widely held according to which the chief energetic function of the maintenance ration is to supply heat to support the normal body temperature.

Were such the case, it is evident that a feeding stuff would have two distinct energy values, or starch values. Of these, the larger, equal to its fuel value, or metabolizable energy, would express its value for maintenance while the smaller, or net energy value, would represent its value for production. Such a dualistic system would introduce most perplexing complications into all comparisons of rations, but in fact it lacks any adequate experimental foundation. No such sharp contrast has been shown to exist between maintenance and production and the prevention of loss of tissue in the former and the deposition of new tissue in the latter appear to be substantially similar in their energetic aspects.

The writers were the first to show[1] in 1902 that with cattle consuming submaintenance rations only about 60 per cent. of the metabolizable energy of timothy hay actually contributed to the maintenance of the animal, *i.e.* to the prevention of the loss of body substance, while the remaining 40 per cent. simply increased his heat production, which was already sufficient to maintain his body temperature. In other words, it was shown that, qualitatively at least, the relations were the same as in Kellner's experiments on fattening.

Repeated subsequent experiments have abundantly confirmed this result[2]. Together with the almost simultaneous investigations of Rubner and the later ones of Lusk and others on the so-called specific dynamic action of foods they have rendered it evident that, under most conditions, heat production is not an end but an incident of metabolism. The body usually does not metabolize because it must produce heat

[1] U.S Dept. of Agr., Bur. Anim. Indus., *Bull.* No. 51.

[2] Compare *The Nutrition of Farm Animals*, pp. 271–272.

but produces heat because it metabolizes. In his later writings[1] Kellner fully accepted this view and distinguished in feeding stuffs between "Thermic" energy, which can only yield heat in the body, and "Dynamic" energy (equivalent to our net energy), which is available for physiological processes, and pointed out that what is required for maintenance is not a supply of thermal energy equal to the minimum heat loss from the body but a supply of dynamic energy sufficient to support the necessary bodily activities.

Such being the case, it is clear that in maintenance as in productive feeding only a part of the metabolizable energy of the feed is utilized, except perhaps at unusually low temperatures. Whether the proportion which can be utilized is the same in the two cases it is perhaps too early to assert positively. Certain theoretical considerations might lead one to suppose that it would be somewhat greater in maintenance than in production, but within the range of our own experiments[2] the results, although somewhat variable, do not on the whole indicate that the difference can be great and we are inclined to believe that the same starch values, or net energy values. are applicable without material error to estimating the values of feeding stuffs both for the maintenance and the fattening of cattle and presumably of other species of ruminants.

To recapitulate, then, Kellner's starch values represent neither the digestible carbohydrates (actual or potential) contained in feeding stuffs nor the fuel value of the material which they supply to the tissues. What they seek to express in another form is precisely what we have expressed in our net energy values, viz., the extent to which the feed is able, either to diminish or prevent loss of stored energy from the body (submaintenance and maintenance rations) or to bring about a storage of energy in new tissue (fattening, growth, etc.). Aside from experimental errors, there is no difference in principle between the two sets of values but merely a difference in the manner of expression. It may be presumed that whichever of the two is on the whole preferable will survive, but in view of the various senses in which the term starch value may be used we feel that there are at least certain manifest advantages in frankly accepting the fact that we are dealing with the feed as a source of energy and in employing a recognized unit of energy to express its value.

[1] Compare *Ernährung landw. Nutztiere*, 6th ed., pp. 105–109.

[2] *Journ. Agr. Research*, **3** (1915), 472.

(*Received January 17th*, 1919.)

VOLUME IX SEPTEMBER, 1919 PART III

SILVER-LEAF DISEASE, III
(INCLUDING OBSERVATIONS UPON THE INJECTION OF TREES WITH ANTISEPTICS).

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CONTENTS.

1. INTRODUCTION.

THE present paper contains the results of investigations on silver-leaf disease which are a continuation of those previously described by one of us (1, 2) in earlier numbers of this periodical.

Some time ago it was thought desirable to duplicate as far as possible in different localities the experiments conducted on this disease. Hence most of the experiments described in this paper have been carried out both at Cambridge and at the John Innes Horticultural Institution, Merton.

As before, the experimental work at Cambridge has been done on the University Farm by permission of the School of Agriculture, and in the Botanic Garden by courtesy of Mr R. I. Lynch. The trees used at Cambridge were mostly provided by a grant from the Development Fund to which reference was made in earlier papers of this series.

Few papers on silver-leaf disease have appeared since the publication of the paper by one of us (2) in 1913, the most noteworthy being that by Smolak (9), to which it is necessary to devote some attention before our own results are considered.

As pointed out in the *Annals of Applied Biology* (4), he has inadequately represented the views on silver-leaf expressed by one of us in earlier papers. For instance, Smolak states that "according to the investigations of Brooks the basidiomycete *Stereum purpureum* is the cause of silver-leaf disease,": whereas the view put forward was that "silver-leaf disease is a general pathological phenomenon, one cause of which is the fungus *Stereum purpureum*," a view which, it may be said at once, we see no reason to change.

Smolak's observations on the cytology of silvered leaves are interesting, but there is no reason why the changes in the cell contents described by him should not be attributable either directly or indirectly to *Stereum purpureum* present in woody tissues below, even though the influence of a specific enzyme carried up in the transpiration current as suggested by Percival (7) be excluded. Smolak apparently finds it difficult to conceive of action at a distance in the association of *Stereum purpureum* with silver-leaf disease, and suggests (*loc. cit.* p. 154) that silvering is an autolytic action. The presence of the fungus in the woody tissues of the stem may, however, cause some chemical or physical disturbance in the transpiration current, which, upon being felt in the leaves above, causes silvering. Such a disturbance in the transpiration current may either directly bring about the change or indirectly do so by liberating some other substance in the cells of the leaves to which the change is immediately due. Where silvering is not due to *Stereum purpureum* the phenomenon may be caused by the liberation of the same substance in response to other, at present unknown conditions, or may be otherwise brought about by these particular conditions. Even when the primary agent in silvering is *Stereum purpureum*, the last links in the chain of cause and effect are not yet known.

The cytological peculiarities of silvered leaves described by Smolak are possibly incipient stages in the death of the cells slowly induced by a disturbance in the normal physiology of the tissues. No statement is made as to the age of the silvered leaves examined by him.

Smolak also adds that "perhaps the inoculation experiments hitherto carried on have been too few or possibly they are not yet complete enough to decide the relation of *Stereum* to this disease," but in view of the very numerous successful inoculation experiments carried out by

Percival (7), Pickering (8), Güssow (5), and Brooks (1, 2), including many pure culture inoculations performed by the last named, this statement is surprising. Later in the present paper it will be shown that all Koch's postulates have now been carried out and fructifications of *Stereum purpureum* have developed on trees which were inoculated with pure cultures of the fungus. The suggestion of Smolak that silver-leaf may be due to bacterial infection is not supported by definite evidence.

As regards the physical reasons for the appearance of silvering, there seems to be little disagreement between Smolak's views and ours. We still consider that by far the greater part of the peculiar appearance of plum leaves in the earlier stages of silvering is due to the formation of abnormal air spaces below the upper epidermis, whereby the character of the light normally reflected is changed. If the upper epidermis of a plum leaf which has not been long silvered is stripped off, the underlying mesophyll is dark green in colour and is indistinguishable to the naked eye from the mesophyll of a normal leaf. The mesophyll of plum leaves which have been silvered a long time on the other hand does appear lighter green in colour than that of healthy leaves viewed in the same manner. The individual mesophyll cells of young silvered leaves examined freshly under the microscope, cannot be distinguished from corresponding cells of a normal leaf, although leaves in which the silvering is of long standing eventually show a considerable number of cells in which the chloroplasts are fading and disorganising. It is noteworthy that even in a normal plum leaf a few of the cells often show disorganised chloroplasts long before the onset of autumn. Smolak did not examine the contents of silvered cells in the fresh condition.

2. FURTHER OBSERVATIONS UPON THE OCCURRENCE AND INCIDENCE OF SILVER-LEAF.

The leaves of a plum tree affected by *Stereum purpureum* often show silvering both at and directly after the time of unfolding in the spring, but sometimes only the first formed leaves on a particular shoot exhibit the affection. The same is often true of shoots belonging to second or "midsummer" growth later in the year. Although leaves are sometimes completely silvered at the time of unfolding many leaves subsequently become silvered which were normal while expanding. Smolak states (*loc. cit.* p. 141) that "the spreading of the silvering always began first of all on and around the vascular bundles (veins)." As the network of veins in a plum leaf is very intricate and permeates almost the whole of the leaf, this statement does not appear to be clear, but if the principal

veins are meant, it must be pointed out that silvering often proceeds from the leaf margin instead of from one of the larger vascular bundles. With parti-silvered leaves the boundary between the normal and abnormal parts may be sharply defined and may remain fixed, but by marking the boundary with Indian ink, an extension of the silvered area can sometimes be observed.

Since the publication of Brooks' paper on silver-leaf in 1913 (2), numerous examples of silvered foliage occurring naturally in other plants have been seen. Mention will be made first of those in which silvering was associated with the presence of *Stereum purpureum*.

In July, 1913, our attention[1] was called to a number of silvered sloe trees amongst healthy ones in a field hedge near Cambridge. In one of the affected trees some of the branches were dying back. Sections of branches bearing silvered leaves showed sectors of discoloured wood, containing hyphae as described (1, 2) for silvered branches of cultivated plums. The affected leaves exhibited the usual characters of silvered foliage, the tendency of the mesophyll cells to fall asunder being very pronounced. During October of the same year fructifications of *Stereum purpureum* developed in abundance on the dead portions of this silvered tree.

During the summer of 1913 an apricot tree growing against a wall was seen to be silvered and some of its branches were dying back. The leaves showed the usual characters of silvered foliage and the branches that were dying back contained discoloured zones of wood as in silvered plum trees. The silvered branches continued to die during the summer and fructifications of *Stereum purpureum* arose on them during the autumn.

Silvering of laburnum trees associated with *Stereum purpureum* was described in the previous paper of this series (2) and it suffices to say that several other silvered trees of the same kind have since been seen associated with this fungus. The frequency of silvering in laburnums in private gardens is probably due to the pruning to which they are often subjected, the wounds so made affording abundant opportunity for the entrance of the wound parasite, *Stereum purpureum*.

During 1915 many silvered shoots were seen arising from stools of poplar trees and on each of the five stumps from which such shoots arose, *Stereum purpureum* was growing abundantly. These stools formed part of a row of poplars, the intact trees of which showed normal

[1] We are indebted to Mr F. G. Tutcher, Sub-Inspector of the Board of Agriculture and Fisheries for pointing out these trees to us.

foliage. In addition to the shoots arising from the stumps, one sucker arising some distance away from them was silvered. Affected leaves were examined during September and fresh sections showed 20–50 per cent. of the palisade cells with disorganising chloroplasts, the sections as usual showing a marked tendency to fall asunder. It is not known how long these leaves had been silvered.

The only other record of silver-leaf in poplars as far as can be ascertained occurs in the report of the Scientific Committee of the Royal Horticultural Society for 1913, when one or two of a number of leaves of a poplar attacked by *Ascomyces* (*Taphrina*) *aureus* submitted to the Committee were said " to show the silvery appearance characteristic of the attack of *Stereum purpureum* as seen in plums."

As regards other plants referred to in the previous paper (2), in which silvering was associated with *Stereum purpureum*, it may be stated that in hundreds of plum trees, especially Victorias, seen in different parts of the country, the association of this fungus with dying silvered branches has been invariable. The character of the soil appears to have little influence on the incidence of the disease in plums, which seems to be as common on soils amply provided with lime as it is where this constituent is less abundant. In addition to Victoria, the variety Czar is also very susceptible to silver-leaf in some districts, but other varieties, e.g. Monarch, Early Rivers, Pond's Seedling, and Pershore are only occasionally attacked. The relative immunity of the large mature areas of the last-named variety in the Evesham district is very striking. A belief prevails there that the variety Victoria worked on the Pershore stock instead of on other stocks generally used, e.g. Brampton, is thereby rendered relatively immune from silver-leaf disease. There is, however, no definite evidence yet that this belief is well founded and certain inoculation experiments described below do not support it. Occasionally *Stereum hirsutum* as well as *Stereum purpureum* is seen on silvered plum trees, but as described under the heading of inoculation experiments, the former cannot be considered a cause of silver-leaf.

With silvered apple trees, the presence of *Stereum purpureum* is by no means so constant as in plums except possibly in regrafted trees.

A silvered cherry tree near Cambridge came under observation in 1913, several of the diseased branches containing discoloured wood, but it is not known whether *Stereum purpureum* subsequently developed upon it. Another silvered cherry tree was seen in the North of England in 1916. As will be shown later, silvering has been induced in old cherry trees by inoculation with *Stereum purpureum*. Some cherry trees about

ten years old which were cut back in 1915, showed silvered shoots arising near the unprotected extremities during 1916. Examination showed that the portions bearing the silvered shoots contained much discoloured wood whereas in parts similarly cut back and bearing healthy branches there was very little discolouration of the tissues.

Several additional silvered red currant bushes have been seen on which *Stereum purpureum* developed.

Silver-leaf is of common occurrence in the Portugal laurel and it has often been seen in connection with dying back of the branches. Other observers have seen *Stereum purpureum* in association with the disease on these plants.

On the other hand, examples of trees killed by *Stereum purpureum* with which the phenomenon of silvering was not associated, have been seen. Since 1913, when brief reference (2) was made to this possibility, certain birch and plane trees growing in gardens, after being severely cut back, have been rapidly killed by this fungus without silvering of the leaves being evident.

In 1913 (2) it was pointed out that several examples of silvered foliage had been seen with which there was no connection with *Stereum purpureum*.

Since that year numerous other examples of silvered foliage have come under observation which could not have been induced by *Stereum purpureum*, and with which there could have been no connection.

In 1913 (2) the silvering of certain batches of seedling plums was described, and it was pointed out that, though this affection was strictly comparable with the silvering of adult trees, it was certainly not attributable to *Stereum purpureum* as no fungus mycelium was found in the tissues.

Since then several other lots of seedling plums and other seedling plants have been examined and many of them have shown silvering. The affection, if it is really an affection in these cases, is capricious in occurrence. Of batches of seeds treated apparently in the same manner, some may give rise to silvered seedlings while others develop normally.

The subsequent history of the silvered plum seedlings raised by Mr W. O. Backhouse and described in 1913 (2) will first be given. Before planting out in the open during May, 1912, several of these silvered seedlings were examined microscopically and although sections of the leaves showed the usual tendency to fall asunder, no trace of fungus attack could be found in stem, leaf, or root. During the summer and early autumn of 1912, the condition of these silvered seedlings

seemed to show a gradual improvement, i.e. their foliage appeared to become more normal in appearance. During February, 1913, these seedlings, then about a foot high, were cut down in order to be used for scions. In May, 1913, about fifty of these 400 Victoria seedlings were still silvered and by 1914 only about ten remained silvered, so it is clear that these plants gradually grew out of the affection.

While visiting the Agricultural Research Station at Long Ashton, Bristol, during July, 1913, Professor Barker called the attention of one of us to the presence of silver-leaf in seedlings of certain kinds of *Ribes*, *Rubus*, and *Prunus*. These seedlings had been planted out in May of the same year in soil which had been broken up from grass land two years previously and had not been manured. The following is a summary of the observations made at the time of the visit:

Red Currant (*Raby Castle*).
Silvering common but more marked in old than in young leaves.

Red Currant (*Sweet Red*).
Plants appear to be growing out of silvering.

Red Currant (*Ogden's*).
No sign of silver-leaf.

White Currants (*White Dutch Cut Leaf*).
Plants appear to be growing out of silvering.

Rubus innominatus Kuntzeanus.
Numerous plants with silvered foliage.

Loganberry.
Silvering present but not conspicuous.

Prunus spinosissimus.
One of the five plants was markedly silvered.

It was not possible to examine these silvered plants microscopically, but to the naked eye they appeared to be typically silvered, although most of them were growing out of the affection.

After treatment in the usual manner[1] other batches of plum seeds of different varieties (Victoria, Monarch, Greengage) gathered in 1913

[1] These seeds were treated in the following manner: the plum stones were placed in damp soil as gathered and when the collection was complete the stones were washed and then immersed in shallow boxes of sand previously dry heated to 100° C. The boxes were then placed in a cold frame and kept damp. Early in January the seeds were taken from the stones and then planted in soil which had been treated with toluol and placed in a greenhouse from which frost was excluded. Under these conditions germination proceeded.

were sown under varying conditions (in ordinary soil, in manured soil, in sand, and at different distances apart) during the winter of 1913–14, but none of these seedlings showed characteristic silvering although the foliage of a small number developed a peculiar leaden pallor. Some of the Victoria seeds used in this series were obtained from heavily silvered trees. Large numbers of red currant seedlings (from seed gathered in 1913) grown in boxes also developed normal foliage.

Other seedlings of various kinds grown under similar conditions by Mr G. O. Sherrard at the John Innes Horticultural Institution showed different results. Thus several seedlings of the following varieties of plums, Denniston's Superb, Early Transparent, and Gisborne showed silvered foliage, and nearly all the seedlings of loganberry and "Phenomenal Berry" possessed silvered foliage. On the other hand seedling apples and nectarines had normal foliage. The seedling *Rubi* were planted out in the open and showed a considerable recovery by the autumn.

These observations show that silver-leaf occurs commonly but irregularly in certain seedling plants notably plums, and species of *Rubus*. In these cases the silvering does not appear to be of pathological significance as the seedlings grow out of it in time. Nothing is yet known as to the cause of silvering in these seedling plants but our observations indicate that it is not due to a parasitic organism.

It was pointed out in 1913(2) that plants of the white dead nettle with silvered foliage had been observed, and since then we have seen, during the latter part of the winter and early spring, many kinds of herbaceous plants with silvered foliage including *Lamium purpureum*, *Lamium amplexicaule*, *Veronica agrestis*, *Raphanus sativus*, *Urtica dioica*, *Plantago major*, rhubarb, and *Primula spp.* Mr G. Lamb writing in the *Gardener's Chronicle* for November 15th, 1913, recorded silvering of tomato foliage.

Several of the above plants have been microscopically examined by us and like the white dead nettle described in 1913 showed the typical characters of silvering. In these herbaceous plants there appears to be no regularity in the distribution of silvered leaves upon an individual plant, some branches bearing silvered leaves and others normal leaves. Fresh sections of a silvered leaf of *Lamium purpureum* showed no noticeable difference as regards the chloroplasts from those of a healthy leaf, although the epidermis was loose and there was the usual tendency of the mesophyll cells to fall asunder.

Two other examples of silver-leaf may be noted. Mr F. J. Chittenden

of Wisley informed one of us in 1913 that he had seen "a piece of a *Lonicera* (? *japonica*) with foliage showing all the characteristics of silver-leaf."

In 1914 one of us saw a single elm leaf on a small branch arising about seven feet up the trunk of a large tree, the middle of which was markedly silvered with the exception of some dark green flecks although the margin was either dark green or white. Other leaves of the same branch showed variegation but not silvering.

Finally, a few other leaf conditions may be noted, which, though not silver-leaf in the sense described above, simulate it closely.

While one of us was in Malaya during 1914 a branch of the Borneo camphor tree (*Dryobalanops aromatica*) came under observation which appeared as if silvered. The foliage of this plant is normally dark green but this branch had been severed from the tree and had been lying exposed to the sun for a few hours, after which the foliage appeared to be silvery. The leaves of the plant are too stiff to wilt and it seemed as if the upper epidermis had broken away from the mesophyll, thus allowing air to accumulate below the epidermis and so altering the character of the reflected light.

In the *Journal* of the Board of Agriculture for 1913 one of us(3) recorded the occurrence in the south of France of several kinds of shrubby plants, notably *Arbutus unedo*, *Myrtus communis*, *Viburnum Tinus*, with "silvery" foliage in contrast to the dark green leaves which these plants usually possess. Large numbers of these "silvery" plants were seen in irregular groupings, and on casual examination one would have certainly declared that these plants were silvered. Careful observation of leaves in an intermediate condition showed, however, that the "silvery" appearance was due to innumerable punctures of the epidermis by some insect, the character of the light reflected from these leaves being changed in consequence. In this country pea Thrips sometimes affects the pods in a somewhat similar manner.

3. INOCULATION EXPERIMENTS.

(a) *Experiments with plum trees.*

Large numbers of inoculation experiments, in addition to those described in previous papers of this series have been performed, especially with *Stereum purpureum*.

During 1911-12 certain inoculations with spores of *Stereum purpureum* were successfully carried out for the first time. Other spore

inoculations performed in a somewhat similar manner since then have given negative results. Thus branches of young Victoria plum trees were snapped across and after being left in this condition for some months, spores were inserted in the wounds without inducing silvering. Other branches were cut off to within two inches of the main stem and after leaving the ends exposed for various periods, spores were inserted, also with negative results.

Inoculations with spores of *Stereum purpureum* carried out, however, under conditions more favourable for infection have nevertheless been followed by silvering of the foliage in a considerable number of cases. The method of inoculation was as follows:

The main stem of a young healthy plum tree was cut back, and a little above the exposed surface a sporophore of *Stereum purpureum* was suspended from a pin after an interval during which the surface was left open to the weather or protected from it by means of a tube. A wad of damp cotton wool was placed over the sporophore to cause it to deposit spores freely, and the whole was enclosed in a glass tube fastened to the top of the stem by means of plasticine. With this arrangement, a heavy spore deposit fell on the end of the stem under conditions favourable for germination and infection. The experiment was varied in different ways—the end of the stem was sawn and covered with gas tar or Stockholm tar in some cases, in others the sawn or smoothed surface was left without treatment by an antiseptic. The following are details of the experiments carried out during 1913–14.

(1) The main stem of a standard Victoria tree 3 4 years old was cut back with a saw to within two feet of the ground on December 2nd, 1913, and the surface covered with gas tar. This remained exposed until January 31st, 1914, when a sporophore of *Stereum purpureum*; taken from a silvered plum tree, was placed over it and covered with a sterilized tube as described above. The fungus was removed two days later and the tube replaced. On May 13th a branch arising an inch below the inoculated surface, was heavily silvered although other branches below this were normal. By July other branches had become silvered and by the middle of September the whole tree was affected.

(2) A standard Czar, 3-4 years old, treated as in (1) except that the cut curface was left untouched. On May 13th, 1914, a branch arising $2\frac{1}{2}$ inches below the place of inoculation was silvered and the cut surface was gumming and covered with a greyish mycelium. Silvering did not extend much in this tree, only one other branch becoming affected before the autumn.

(3) A standard Czar, 3–4 years old, treated as in (1) but the cut surface was covered with Stockholm tar. On May 13th, 1914, a branch 4½ inches below the cut end was silvered although a branch three inches nearer was normal. Silvering did not progress further during the summer.

(4) A standard Czar, 3–4 years old, treated as in (1) but the cut surface was made smooth with a knife and left exposed until inoculation. On May 13th, 1914, silvering was evident on a branch 1½ inches below the cut surface which was gumming. Silvering spread during the summer until nearly the whole tree was affected.

(5) A standard Czar, 3-4 years old, was cut back with a saw December 2nd, 1913, and left untreated. Inoculation with sporophore material of the same gathering was not performed until March 2nd, the fungus being removed and the tube replaced the following day. This tree remained unsilvered.

(6) As in (5) but the cut surface was covered with gas tar. The tree remained unsilvered.

(7) As in (5) but the cut surface was covered with Stockholm tar. The tree remained unsilvered.

(8) On December 13th, 1914, the main stem of a standard Victoria tree 3-4 years old, was cut back with a saw, under sterile conditions, to within two feet six inches from the ground, and the cut surface was immediately covered with a sterile tube embedded in a collar of plasticine. A sporophore of the same gathering as before was inserted on March 2nd, 1914, and was removed the following day, the tube being replaced. In this experiment and in the next two, the inoculations were carried out under conditions as sterile as possible. On May 13th silvering was apparent in two branches eleven and eighteen inches respectively from the cut surface which was gumming. Other branches nearer the cut surface but with a different orientation were unsilvered and remained so throughout the summer.

(9) As in (8) except that the cut surface of the main stem was made smooth with a sterile knife. On May 13th two branches about eight inches below the cut surface were silvered, although two branches nearer the top were normal. By the first week of June all branches showed some silvering which became more marked as the season advanced.

(10) As in (8) except that the upper part of the main stem was half sawn, half broken across. On May 13th some of the leaves on the uppermost branch arising six inches below the cut surface were silvered, like-

wise the leaves of another branch somewhat lower. At the same time the end of the main stem was gumming. Silvering spread only slightly during the summer.

(11) As in (8) but kept as a control. On May 13th no silvering was apparent although the end of the stem was gumming; silvering did not develop later in the year.

Thus, apart from the control experiment, only three of the other inoculations failed to be followed by silvering. Each of the three inoculations carried out under conditions as sterile as possible, resulted in silvering although in one of them it was not the branch nearest the place of inoculation that became affected. In view of the experiments described below in regard to the cutting back of young plum trees without subsequent inoculation and which always remained healthy it is difficult not to connect the development of silver-leaf in the above trees with inoculation by spores of *Stereum purpureum*. Tree (No. 1) was cut down to the ground after the autumn of 1914 and the main stem was found to contain extensive discoloured zones of wood down to the base. Another feature of these experiments is the apparent lack of protection afforded by tar after exposure to the weather for two or three months.

Another series of experiments of the same kind was carried out during the winter of 1914–15.

The following are the details:

(1) The main stem of a bush Victoria, 4–5 years old, was cut back on November 20th, 1914, the surface being made smooth with a knife. On April 19th, 1915, a sporophore of *Stereum purpureum* from a silvered plum tree was suspended over the cut surface and covered with a tube as in the experiments of the previous year. The fungus was removed a few days later and the tube replaced.

Silvering did not develop during the following summer.

(2) As in (1). No silvering.

(3) As in (1). No silvering.

(4) As in (1). No silvering.

(5) As in (1), but the cut surface was covered with white paint. No silvering.

(6) As in (5).

(7) As in (1), but the cut surface was covered with Stockholm tar. No silvering.

(8) As in (7), but the cut surface was covered with Stockholm tar. No silvering.

(9) As in (7). No silvering.

(10) As in (7). No silvering but there was gumming at the cut surface.

(11) As in (1), but the cut surface was charred with a hot iron. During the early summer silvering developed in a branch four inches below the cut surface and later in the season on another branch two inches lower. This tree recovered during 1916.

(12) As in (11). No silvering.

(13) As in (1), but the cut surface was covered with gas tar. During the summer one shoot arising three inches below the cut surface became heavily silvered, and other branches became affected later in the season. In 1916 the tree was generally silvered. In 1917 the tree began to die back and *Stereum purpureum* developed on the dead branches.

(14) As in (13). No silvering.

(15) As in (13). No silvering.

(16) As in (13). No silvering.

(17) As in (1), but the cut surface was covered with white paint and varnish. No silvering.

(18) As in (17). No silvering.

(19) A bush Victoria as before, cut back and tubed April 19th, 1915, under sterile conditions but not inoculated. A small amount of gum developed on the cut surface during May, but there was no silvering.

In addition, three other trees of the same kind were left wholly untreated.

In this series of experiments only two inoculated trees became silvered during 1915. This small proportion of successful inoculations may be accounted for, probably, by the much later date at which the inoculations were performed than in 1913 14.

As will be seen later, there is evidence that inoculations with *Stereum purpureum* are the more effective the longer they are carried out before the growing season.

A series of inoculation experiments will now be described in which portions of sporophores of *Stereum purpureum* were inserted into the wood of young Victoria plum trees at different dates from the middle of February until June. These experiments were conducted because it seemed from previous experience that the fungus was less likely to cause infection if inserted during a period of active growth of the host. Victoria plum trees were inoculated twice a week, one tree on each occasion, from February 16th to May 16th, and after that at weekly intervals until June 2nd. Three branches on a tree were usually inoculated.

The *Stereum purpureum* used for inoculation was obtained from a silvered plum tree about two months before the experiments began and was kept dry in the meantime. At the commencement of the inoculations the sporophores gave a copious spore deposit after being moistened, but as time went on the period required for the deposition of spores increased, so it was considered advisable to use larger pieces of sporophore in the inoculations in order to compensate for the possible diminution of vitality. All trees inoculated until April 26th became heavily silvered during the summer, but after that date, silvering developed only slightly or not at all in the inoculated branches, and in the latter case there was no change the following year.

This result is to be partly attributed to lessened vitality in the fungus, but also perhaps in part to greater resistance of the host during the period of active growth.

A number of inoculations with sporophores of *Stereum purpureum* have been performed by placing the fungus in the bark only and not in the wood. In other sporophore inoculations, the inoculating material has been placed in a wound made into the wood.

Of nine Victoria plum trees inoculated in this manner one winter six became silvered the following spring, and examination of the affected branches showed that the fungus had spread extensively into the wood from the bark. As was to be expected the progress of silvering was at first slower than in trees inoculated directly in the wood.

Another series of inoculations of young trees with portions of the sporophores of *Stereum purpureum* may be mentioned. In March, 1918, fifteen young Victoria trees were inoculated in this way and by July all of these, with one exception, had become silvered, whereas none of the controls became silvered.

Sporophores of *Stereum purpureum* from different sources—silvered plum trees, dead beech trees, and willow stumps have all been used for inoculation, but as the experiments published in 1913 (2) show, there is no difference in infectivity between *Stereum purpureum* obtained from sources associated with silver-leaf disease, and that obtained from woody material which has not been connected with this affection. As long as the sporophore is vigorous it almost invariably produces silver-leaf by inoculation of a Victoria plum tree whatever the origin of the fungus may be.

During 1915 some material of *Stereum purpureum*, from a silvered cherry tree and from broom bushes respectively, was sent to one of us from New Zealand by the kindness of Miss Piggott of Victoria College,

Wellington. Although this material gave no spore deposit on arrival it appeared otherwise to be in fair condition so it was used during August, 1915, to inoculate two Victoria plum trees, but the fungus was inserted too late to expect silvering to result the same year. Profuse gumming occurred at each place of inoculation later in the summer and in the spring of 1916, one of the branches inoculated with the fungus from a silvered cherry tree was silvered and another inoculated branch subsequently became affected, the two remaining inoculated branches of this tree remaining healthy. Neither of the four inoculations made in the other tree from New Zealand material obtained from broom bushes resulted in silvering. Although few of these inoculations were successful, probably on account of the low vitality of the sporophores, the results show that *Stereum purpureum* obtained from New Zealand is capable of causing silver-leaf in plum trees in this country and from this one would suspect that *Stereum purpureum* was a cause of silver-leaf in fruit trees in New Zealand.

Since paper II of this series (2) was published, many additional inoculations with pure cultures of *Stereum purpureum* have been performed and a large number of them, especially in Victorias and Czars, have been followed by silvering.

Pure culture inoculations of the Pershore plum are of some particular interest for it is well known that this variety is only very occasionally attacked by silver-leaf disease under natural conditions. Nevertheless, Pershore trees were readily infected with silver-leaf disease by inoculating their branches with a vigorous pure culture of *Stereum purpureum*. Young Victoria trees growing on Pershore stocks as easily succumbed to silver-leaf by the use of pure cultures of the fungus as Victorias growing on the usual stocks.

As regards relative infectivity of the pure culture, in our experiments it was immaterial in general whether the culture was derived from *Stereum purpureum* growing on a silvered tree or on some other substratum such as a dead willow stump. In carrying out experiments with pure cultures it is important that the fungus should be growing vigorously and that the inoculations should be performed during the winter or early in the year, otherwise silvering may fail to develop.

A considerable number of plum trees which became silvered after inoculation with sporophore or pure culture material of *Stereum purpureum* developed fructifications of *Stereum purpureum* as the trees were killed.

With Koch's postulates thus carried out, it is difficult to look upon

such a result except as a convincing proof of the causal connection of *Stereum purpureum* with silver-leaf disease in these cases. As has been pointed out previously, the fructifications of *Stereum purpureum* usually only develop on the stems of silvered trees as they die back, and it is unusual for the fungus to form its fruit bodies earlier than a year after inoculation although, where infection was particularly rapid, *Stereum* appeared occasionally within a shorter period. In one tree *Stereum* developed within seven months of inoculation but in this instance the fungus arose exceptionally on one side of a branch which had been killed by the fungus while the other side of the branch was still healthy.

Spencer Pickering (8) has shown that plum trees which have recovered from silver-leaf are as liable to re-infection as trees which have not previously been infected by the disease. Our experiments in this connection gave the same result.

Attempts have been made to induce silvering by inoculating leaf stalks with small portions of the sporophores or with mycelium grown in pure culture. Nearly every leaf inoculated in this manner and most of the leaves wounded as controls, fell off soon after the operation, but the few leaves which remained attached after inoculation did not become silvered. Leaves of both plum (Victoria and Monarch) and apricot were used for this purpose.

Many inoculation experiments on plum trees with other species of *Stereum* in addition to those described in 1913 have been performed but with the exception of two doubtful successes with *Stereum spadiceum* (one sporophore, the other pure culture material) without silvering being induced. In both of these, the inoculated trees were old, and it was not possible to trace definitely the connection between the inoculation and the incidence of silvering. A suspicion exists that these trees became naturally infected.

None of the inoculations of young plum trees carried out with *Stereum rugosum*, *Stereum hirsutum*, and *Stereum spadiceum* were followed by silvering although there was sometimes a certain amount of gumming at the places of inoculation. In these experiments both natural sporophores and pure cultures were used but with the same negative result. Some of the branches inoculated in this way were subsequently cut up, and investigation showed that the fungus had spread in the tissues much less than when *Stereum purpureum* was the species used for inoculation.

(b) Experiments with apple trees.

As recorded in 1913 (2) few inoculations of apple trees with *Stereum purpureum* have resulted in silver-leaf. Three other trees of the Lord Suffield variety became silvered after inoculation with sporophores of *Stereum purpureum*, but other varieties (Lane's Prince Albert, Stirling Castle, Bramley's Seedling) inoculated either with sporophore or pure culture material of this fungus or with sporophores of *Stereum hirsutum* have remained unsilvered.

Reference has already been made (2) to the frequency of silvering in scions of regrafted apple trees in fruit plantations. We have had apple trees regrafted in order to keep them under observation in this connection: seven Bramley Seedling trees, and five Stirling Castle trees about five years old were cut back and regrafted in different ways with scions of Bramley Seedling either at the end of the main stem or in lateral branches.

An interval of a month elapsed between the cutting back of the stocks and the insertion of the scions which had been heeled into the ground for some time before use. After insertion of the scions, all exposed surfaces were covered with grafting wax. Most of the scions developed and formed healthy shoots during 1913 and 1914 but one of them became silvered during the summer of 1915. At the same time four old apple trees (two Codlin, one Warner's King, one French Crab) were regrafted with scions of Bramley Seedling. It is worth while to consider these trees in detail.

(1) *Codlin.* Seven scions of the twelve inserted grew well during 1913 the foliage being normal. By June, 1914, however, some leaves of each scion were silvered and the affection became more clearly marked as the summer advanced. During March, 1915, the upper parts of this tree bearing the scions were cut off in order to see if there was any considerable discolouration of the wood. This was present in quantity and up to distances of nearly a foot from the point of insertion of the scions.

There were several places from which the disease may have spread in the tissues. The exposed ends of the stock after the grafting wax had fallen off, dead scions, and the scars made by cutting away lateral branches below the scions, were all centres from which a fungus may have spread into the neighbouring tissues. The zones of discolouration relating to these areas often ran into one another and it was impossible

to determine with which of these the greatest amount of diseased wood was associated.

Portions of the affected branches were cut up and placed on moist sand and fructifications of *Stereum purpureum* developed on several of them within two months. During the summer of 1915, *Stereum purpureum* developed in abundance on the cut end of the stump of this tree remaining in the ground and nearly all the shoots arising from it were silvered.

(2) *Warner's King.* Fifteen scions were inserted in various ways and, of these, ten developed normally during 1913, the other five dying. During 1914 six of these living scions became silvered, the foliage of the others remaining normal. Portions of the tree were cut down in March, 1915, and the distribution of discoloured wood investigated, with the same result as in the tree just described. *Stereum purpureum* developed on some of these portions after being kept on moist sand for two months.

(3) *Codlin.* Eleven scions of Bramley Seedling were inserted, of which six developed normally during 1913 and 1914, the others dying. During 1915 the foliage of five of the scions became silvered.

(4) *French Crab.* Nineteen scions of Bramley Seedling were inserted of which thirteen developed normally during 1913, 1914, and 1915, the others dying.

In these regrafted trees the evidence points to *Stereum purpureum* as the cause of silvering, though it is by no means certain that this fungus is always the cause of silvering in regrafted apple trees.

It is obvious that, in regrafting operations, there is considerable opportunity for the entrance of a wound parasite like *Stereum purpureum,* but regrafted apple trees which become silvered often rapidly grow out of the malady.

In the trees referred to above, the character of the silvered leaves was the same as in plum leaves typically silvered. Thus the upper epidermis could be readily stripped off and the mesophyll cells had a tendency to fall asunder when sections were cut.

(c) *Inoculation experiments with other plants.*

Other kinds of woody plants have been inoculated with *Stereum purpureum* to see whether silver-leaf could be induced in them by this means. The following have been inoculated with success by inserting into the stem small pieces of sporophores: cherry (old trees), red currant (fructifications of *Stereum purpureum* subsequently developed on one

of the silvered bushes), horse chestnut, Philadelphus, and in gooseberry bushes by using mycelium grown in pure culture, although with these plants (cherry trees excepted) the percentage of successful inoculations was much less than in plum trees. Where inoculation was followed by silvering it was found that the fungus made considerable progress in the tissues, but in the unsuccessful inoculations the fungus grew very little. In 1913 certain inoculations of young cherry trees were described and it was pointed out (2) that the small branches experimented with were usually killed outright without an opportunity for silvering to become manifest, but in the successful inoculations now mentioned older branches were used for the experiments with the result that several became silvered.

On the other hand, the following plants have been inoculated with *Stereum purpureum* without silvering resulting: black currant, beech, elm, sycamore, cherry laurel and Portugal laurel. As regards the laurels, other species, e.g. *S. rugosum* and *S. spadiceum*, have also been used as the inoculant, but without success. All these plants, on several of which silvering has been occasionally seen in nature, were young and of vigorous growth and possibly therefore particularly resistant to invasion by the fungus. Inoculated branches which were examined showed that the fungus had made little advance in the tissues.

4. ATTEMPTS TO INDUCE SILVERING OF FOLIAGE OTHER THAN BY INOCULATION WITH *STEREUM PURPUREUM*.

Various attempts have been made to induce silver-leaf in plums without the intermediary of *Stereum purpureum*, and although these have given generally negative results some are worthy of mention.

Attention was first directed to the possibility of inducing silver-leaf by injecting into the woody parts of healthy Victoria plums a sterile extract of wood known to be attacked by *Stereum purpureum*, the assumption being that the extract would be carried up to the leaves by the transpiration current where it might cause silvering. The extract was rendered sterile, except for the minutest viruses, by filtering it through a Chamberland bougie. The injection was carried out in the field by two different methods:

(*a*) In the lower part of the main stem by boring a hole, in which a cork traversed by a glass tube was placed, the latter being connected by rubber tubing to a reservoir situated three to four feet above and containing the extract. The joints were sealed with plasticine and air was excluded from passing into the stem.

(*b*) One of the main roots was severed and the cut end was immediately connected by tubing to a reservoir placed as above. This was found to be the most satisfactory method.

Apart from one tree, silvering was not induced by injecting an extract in this way, and the results being almost entirely negative, one is dubious about claiming that this single tree became silvered on account of injection.

Cut shoots of healthy Victoria plums placed in a similar extract during March and kept as far as possible under sterile conditions, developed their buds normally and the leaves did not become silvered.

Various other substances have been injected into Victoria plum trees in the hope that thereby silvering might be induced, but without success.

Efforts have also been made to induce silvering by checking or otherwise altering the transpiration current. Thus young Victoria plum trees have been drastically root-pruned, branches have been snapped nearly asunder during spring, and the stems of young trees have been cut back so as to leave only a few buds to develop, without silver-leaf developing.

5. FURTHER OBSERVATIONS ON THE MANNER OF INFECTION IN FRUIT PLANTATIONS.

Our views in this respect have undergone no material change since the publication of the second paper of this series in 1913, in which by far the most important cause of silver-leaf disease in the fruit plantations of this country was considered to be the fungus, *Stereum purpureum* acting as a parasite which entered through wounds in the stems and branches of the trees.

The possibility of root infection was not excluded where diseased roots came in contact with healthy ones or where roots became exposed and wounded from some cause or other, but we have not yet observed any trees, which indubitably became infected in this way. Wounds made during grafting or budding are an obvious source of danger. Plum trees in an advanced state of the disease often throw up suckers which are silvered on account of the passage of the fungus into the subterranean parts and Hector (6) has stated that in some parts of Middlesex such silvered suckers have been used as stocks upon which Victoria and other varieties were "worked." It is clear that if a silvered sucker used as a stock contained discoloured wood bearing the mycelium of *Stereum purpureum* there would be a probability that the scion would also become attacked. It by no means follows, however, that silvered

suckers arising from diseased trees necessarily contain discoloured wood themselves and, if they do not, there is no reason why these suckers used as stocks should not grow out of the malady.

The practice of using silvered suckers as stocks cannot be condemned too strongly, but it is not one which reliable nurserymen would follow under any circumstances. In our own experience we have only rarely seen plum trees under five years of age silvered and the danger of the disease being spread from reputable nurseries in the manner described above is probably negligible.

It is not usually until a plum orchard is in full bearing that silver-leaf plays havoc on an extensive scale even with the most susceptible varieties. From the age of twenty years onwards is the most critical time, and, as has been pointed out in the earlier papers, single branches of trees are usually first attacked, the earliest trees to become affected being scattered in the plantation.

If the first trees to be attacked are neglected and allowed to die back with the result that fructifications of *Stereum purpureum* develop, other trees adjacent to these may fall a prey with the result that groups become affected and simulate an attack by a root parasite which spreads through the soil.

As is well known, the variety Victoria is the most susceptible to silver-leaf disease of all plums grown in this country, and perhaps the variety Pershore is the most resistant. It has been thought that the former variety was particularly susceptible perhaps on account of the frequent breaking of the branches through overloading with fruit, thereby providing wounds for the fungus to enter. This idea is, however, invalidated by the remarkably brittle character of the old branches of the Pershore plum, trees of which are often built up, as it were, anew, by the development of young branches from the lower parts of the tree. In mature plantations of Victoria and Pershore plum there would probably be more wounds available for *Stereum purpureum* in the latter than in the former. The explanation of the special susceptibility of the variety Victoria is probably more subtle and is more likely to be found in some difference in the character of the wood itself. In horticultural parlance, the Victoria is known as a soft-wooded, and the Pershore as a hard-wooded variety, and this difference in texture, which signifies much, is probably concerned in this matter.

6. EXPERIMENTS ON THE CURATIVE TREATMENT OF SILVER-LEAF DISEASE.

It was pointed out in 1913(2) that in the state of our knowledge then, the only practicable means of control of the disease in fruit plantations lay in the excision of silvered branches and the eradication of trees which were beginning to die back. These measures and the results obtained by their application on a large scale will be referred to later in this paper. The present section deals with experiments of a chemical or quasi-chemical nature made in an attempt to seek a cure by medical rather than by surgical means.

Various workers have stated from time to time that they have cured silver-leaf in fruit trees by certain forms of manurial or chemical treatment, but upon enquiry it has been found that the number of trees so treated has been small and sometimes one only. It is also now well known that silvered trees not infrequently recover without treatment of any kind, so that the value of experiments conducted with small numbers of trees, especially where adequate controls are not kept, is doubtful. The extent of natural recovery varies considerably from year to year, but in our experience it was most marked in 1915 when a considerable number of the plum trees used in these investigations recovered naturally.

For the most part, the plum trees experimentally treated as described below, were in a moderately silvered condition as a result of inoculation by *Stereum purpureum*, although none of them had begun to die back at the time of treatment.

(*a*) *Treatment with artificial manures.*

In the *Gardener's Chronicle* for August 16th, 1913, "Southern Grower" stated that the application of artificial manures had caused the partial or complete recovery of several plum trees in one of his older plantations. During the winter of 1913–14 the root systems of five silvered trees in our plots were treated each with two pounds of a mixture of two parts superphosphate, one part sulphate of ammonia, and one part muriate of potash, the amount applied being less than that used by "Southern Grower" as the trees were younger. In 1914 none, and in 1915 one, of these trees recovered, although in the latter year several other untreated trees became healthy.

Four other trees heavily dressed with farmyard manure, two receiving a second application, showed recovery only in one.

Of five additional trees treated with both farmyard manure and two pounds of sulphate of iron each, applied to the roots, one recovered two years after treatment. The use of sulphate of iron in this connection will be referred to shortly.

Thus there does not appear to be any reasonable prospect of amelioration by manurial treatment, and, as is well known, the free use of nitrogenous manures often has the effect of making plant tissues specially susceptible to fungoid diseases.

(b) *Treatment with sulphate of iron.*

The application of this substance, both as a dressing applied to the roots and by plugging it into the stems was dealt with in the previous paper of this series (2). The roots of four additional trees were treated each with two pounds of this substance, two of them receiving a second application, but all became worse instead of better. The experiments in plugging silvered plum trees with sulphate of iron carried out by Mr E. Neaverson near Wisbech, were described in a preliminary way in 1913 (2), and through his kindness we are enabled now to give the final results of this experiment conducted by him. Of thirty-seven slightly affected trees plugged in 1910, twelve recovered during 1911, but by 1917 all of these had again succumbed to the disease some, indeed, so badly, that they had been felled together with others which had not recovered after treatment. Badly silvered trees plugged at the same time showed no recovery. As some slightly silvered trees in the same plantation also recovered without treatment, the above figures indicate that the application of ferrous sulphate to silvered trees in this way is not likely to be successful. When the treated trees were felled, it was ascertained that the sulphate of iron had disappeared and had presumably been absorbed into the tissues. Its toxic properties would doubtless kill some of the fungus in the wood though probably only in a limited zone. To ensure all parts of a tree containing the mycelium of *Stereum purpureum* being permeated by a solution of ferrous sulphate sufficiently strong to kill the fungus without seriously affecting the living cells of the tree would be very difficult, and is likely to be more completely effected by absorption through the roots than by the method just described.

(c) *Treatment with the fruit-bodies of Coprinus.*

In 1913 Miss Baker announced in the *Annals of Botany* that she had cured a silvered branch of a Victoria plum by injecting into it a

concentrated aqueous extract of the deliquescing fruit-bodies of *Coprinus*, the idea prompting this treatment being that the mycelium of *Stereum purpureum* might be digested, as it were, by the extract seeing that the fructifications of *Coprinus* undergo autodigestion. Miss Baker carried out only one experiment in this connection and there was no "control." To test this matter further, six silvered trees were injected by us with ripe fruit bodies of a species of *Coprinus* during 1913, holes being bored into the main stems, in which the fungus was inserted and sealed up. In the following year none of the trees had recovered and two were in a dying condition. In 1915 two of the treated trees recovered, but it is to be noted that this was the year in which a large number of healthy untreated trees likewise regained a healthy appearance.

(*d*) *Treatment with other antiseptics.*

Reference has already been made to the plugging of the stems of silvered trees with ferrous sulphate. Other antiseptic substances, e.g. salicylic acid, have been used on a limited scale in the same way, but without success.

In view of Ehrlich's work in medical therapy, the effect of an injection of neo-salvarsan was tried upon two silvered plum trees in the spring of 1914. Holes were bored into these trees towards the base of the stem and ·06 gram of this substance dissolved in 5 c.c. distilled water was poured into each, the holes being afterwards sealed with plasticine. Two injections were made in one tree and one in the other. The tree injected once did not recover, but in the one which was given two doses, the upper leaves wilted soon after the unfolding of the buds and the top of the tree died; this was probably due to the neo-salvarsan being too concentrated. The lower branches of this tree recovered on the other hand, and other healthy shoots subsequently arose. It is likely that the fungus in this tree was killed by the injection of neo-salvarsan, but a considerable part of the tree also was killed in the process.

Preliminary experiments on the injection of dilute solutions of certain aniline dyes into the branches of a large plum tree having shown a possibility of success by treatment with these substances, further trials of a more careful nature were made. For this purpose, the main roots of the trees to be experimented with were exposed at about the time of the opening of the buds; one, two or three of the roots were then severed and at once connected by means of tubing with reservoirs

containing a dilute solution of the dye, placed at a higher level. The exposed portions of roots were enclosed with slates which were readily removable if the roots required attention. At first, absorption of the solutions was rapid, and when it slowed down, the severed ends of the roots were pared, thus allowing absorption again to become vigorous. Dilute solutions of the following dyes were used for this purpose; Congo Red, Gentian Violet, Eosin, Methyl Violet and Methylene Blue. It was not expected that the last-named would prove toxic to the fungus as it is often used in dilute solution for *intra-vitam* staining, but, being a very clear stain, one thought it had the best chance of showing, by staining the wood, whether such dyes were carried to the extremities of the trees. The exact strength of these dyes in the transpiration current cannot be given for obvious reasons, but it was considerably less than 1 in 2000, as the solutions were made up at a strength of 1 in 2000 or 1 in 10,000. The results were as follows:

(1) *Eosin.* Of four treated trees, two recovered although some of the young leaves withered in one of these. A third tree showed partial recovery but some of the leaves were killed. The remaining tree showed no improvement.

(2) *Methyl Violet.* One tree recovered but two others showed no improvement. None of the leaves were killed.

(3) *Congo Red.* The only tree treated with this became healthy. None of the leaves were killed.

(4) *Gentian Violet.* As for Congo Red.

(5) *Methylene Blue.* One tree recovered and one remained silvered. None of the leaves were adversely affected by this dye.

(6) *Control.* One tree in which distilled water was absorbed in the same way did not recover.

In the trees which absorbed these dyes, the xylem of the leaf petioles, especially the lower ones, became stained after a time, methylene blue being most evident, although it could not be detected in the uppermost leaves of the trees. The recoveries in these experiments were more numerous than have ever been observed by us to occur naturally, and it is likely that some at any rate were due to these dyes acting toxically on the fungus without seriously affecting the host. At present, these results are only of scientific interest, and until treatment of this kind has been applied on a larger scale, much importance cannot be assigned to it. From the practical point of view nothing can be done at present along these lines as the methods here used are too troublesome and too costly. If, however, certain of these dyes could be obtained at a cheap

rate, modified methods of injecting trees invaded by fungi in the wood, might be tried with some prospect of success.

While the above experiments were being carried out with dyes, other toxic substances also were injected in the roots of silvered trees in the same way, but with little or no success. Of two trees injected with a weak solution of ferrous sulphate (made up 1 in 1000) one doubtfully recovered and the other remained silvered. Solutions of corrosive sublimate and quinine sulphate (made up 1 in 1000) were without effect. Sodium arseniate, on the other hand, made up at the same strength, had a rapidly toxic effect upon the injected tree and although the strength of the solution was quickly reduced to 1 in 20,000 all the leaves withered except on one branch low down on the side away from the place of injection. These leaves remained silvered but by the following year all the upper part of the tree was dead and only a few sucker shoots, which were healthy, remained.

7. TREATMENT IN FRUIT PLANTATIONS.

In the light of the foregoing statement it will be recognised that there is at present no known *curative* treatment for this disease that can be applied on a commercial scale with prospect of success. The only sound method of control is along the lines advocated in the previous paper and advised in the leaflets recently issued by the Board of Agriculture. Where silvered branches have been properly cut back and dying trees have been grubbed so that *Stereum purpureum* is not allowed to fructify in the plantations, the disease has been kept under control and its spread has been so greatly checked that it has ceased to be a menace in some of the largest commercial plum gardens in the country, in which silver-leaf disease was formerly common. On the other hand, if the fungus is allowed to fructify with impunity the disease spreads with alarming rapidity in Victoria plums.

The following table kindly supplied by Mr E. Neaverson is an illustration of the rapid spread of silver-leaf disease in a Victoria plum plantation near Wisbech, even where conditions were not specially favourable for the extension of the disease. Some of these trees were plugged with sulphate of iron as described earlier in the paper and the most severely affected trees were cut down each winter though *Stereum purpureum* often developed on them before the trunks were removed from the plantation:

	1910	1911	1912	1913	1914	1915	1916	1917	1918
Number of trees standing	154	128	108	100	87	73	67	49	40
Healthy trees	74	78	69	60	50	37	29	23	16
Trees slightly affected	40	21	18	18	17	20	20	15	17
Trees badly diseased	40	29	21	22	20	16	18	11	7

Practical details in regard to the application of the methods here mentioned are given in the leaflets of the Board of Agriculture and need not be repeated here. It is admitted that hygienic measures of this kind are troublesome to carry out but they are commercially profitable and offer the only present means of successfully combating one of the most menacing fruit diseases.

It is likely that any measures which conduce to the general well-being of the trees will tend to reduce the danger of this disease, but these are not sufficient without the application of the means of control outlined above.

Fruit growers must be educated to look upon the fungus *Stereum purpureum* as a dangerous enemy wherever it occurs in the vicinity of fruit plantations, and they must deal with it accordingly.

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(*Received March* 11*th*, 1919.)

NITRIFICATION IN EGYPTIAN SOILS.

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The question of biological activity in Egyptian soils has never received adequate attention. Beyond the fact that nitrification was known to occur readily in Egypt as in most hot countries no series of regular observations has as yet been published dealing with the subject. The following results were obtained during a two years' study of the biological conditions of the soil of an Egyptian farm with a few observations further afield.

The Bahtim Experimental Farm of the Sultanieh Agricultural Society on which most of the work was done is situated ten kilometres north of Cairo and the soil of the farm is typical of the Nile alluvium. Owing to the large head of stock on the farm and the frequency with which fodder crops are fed on the land, the soil is somewhat richer than is usually the case in Egypt, but on the whole the conditions are typical of the normal farming conditions of the southern part of the Nile delta.

Much of the previous work on nitrification has been done under conditions of rainfall sufficient for or even in excess of agricultural requirements. The work of Pouget and Guiraud in Algeria[1] was carried out under a winter rainfall of 189 mm. as against 20 to 40 mm. near Cairo. The conditions in the north of the Delta in the winter months at least must be very similar to those of Algeria and similar results might be expected.

The only reference to nitrification in Egypt is that of R. Roche[2] who pointed out that nitrification goes on readily in Egyptian soils as soon as conditions are suitable.

Egyptian soil temperatures.

One of the most important factors controlling the activity of soil bacteria is that of temperature. During the first year of these observa-

[1] *Comptes Rendues*, 1909, **148**, 725.

[2] *Bulletin de l'Institut Egyptien*, Dec. 1907.

tions reliance had to be placed on the temperatures observed at the time of sampling, supplemented by the records of the Egyptian Ministry of Public Works, which however refer to the subsoil only. A regular series was started at Bahtim in 1918 and records obtained for the surface soil, the thermometer bulb being placed at a depth of 15 cm. The following table gives the monthly means of soil temperatures observed at Bahtim.

Table I.

Monthly means of soil temperatures at a depth of 15 *cm. Bahtim,* 1917–18. *Degrees centigrade.*

Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
21	18	15·4	12·6	14·2	18·3	23·5	25·8	28·2	29·6	28·1	26·1

The biological processes in the soil should not then be normally limited in Egypt by temperature conditions, which at all times are seen to be favourable. Factors limiting such processes as nitrification must be looked for in other directions.

Egyptian agricultural conditions.

Agriculture in Egypt has always depended on the Nile water supply. With the exception of certain coast lands where dry farming is possible during the winter months and where the Bedouins raise a crop of barley, the rainfall is quite insufficient to supply the requirements of even the winter crops. The moisture content of the soil is therefore almost entirely dependent on the conditions of irrigation. The most important Egyptian crops are: Cotton, Maize, Wheat and Bersim (Egyptian Clover). Of these cotton is the most important financially. It is sown in February or March and occupies the ground until October and November. It is essentially a summer crop. Wheat and Bersim are winter crops; sown in October, November or December; they occupy the land until May or June. It is possible, however, to get a single cutting of Bersim just before the cotton crop. Maize is grown with the surplus of water that is available in the time of the Nile flood and is essentially a "Nili" crop. In Upper Egypt millet is grown as an early summer and Nile flood crop and beans as an important winter crop on the basin lands. In addition there are a number of special crops, such as rice, sesame, lentils, onions, henna, which demand special soil conditions and moreover find little place in the average farm rotation of Lower Egypt. Cotton is such a valuable crop that it is grown as frequently as possible, in most cases once in two years; under the best

farming conditions however it would occupy not more than one-third of the land. A typical farm rotation is shown in the following table but many other combinations are possible.

TABLE II.

Farm rotation typical of Lower Egypt. (Three years.)

Cotton: March to October.
Bersim: October to May (four cuttings).
Maize (or Fallow): July to October.
Wheat: November to June.
Maize (or Fallow): July to October.
Bersim (or Fallow): November to January (one cutting).

Maize is not always grown on all the land available, a considerable proportion of the land will then be fallow from June until October as a preparation for wheat or early bersim. The amount of fallow land during the winter months will depend more or less on the quantity of bersim required to feed the farm animals.

So far as Egypt is concerned, no series of experiments have as yet been carried out with the idea of determining the best rotation to adopt; the effect on the soil of the frequent growth of cotton is not yet fully understood. In this connection, two points may be noted however: in the first place the yield of cotton per acre has been declining during the past twenty years and the decrease is not accounted for entirely by the attacks of insects like the Pink Boll Worm which has caused such enormous damage in late years. Even the theory discussed by W. L. Balls[1] concerning the pernicious influence of a gradually rising water table on the cotton crop is probably far from complete.

In the second place, good yields of cotton can be obtained without manuring. This is a fact now generally accepted by the Egyptian farmer and has been well demonstrated by carefully conducted field experiments. The cotton crop in Egypt at the present time is indeed independent of the artificial application of fertilisers[2]. Where definite increments in yield have been obtained, the increase has been too small or too uncertain to repay the cost of the manure.

[1] *Phil. Trans.* 1917, B. **208**, 157.

[2] *E.g.* F. Hughes, "Report on the manurial trials on Cotton carried out during the season 1908," *Yearbook Khedivial Agric. Soc.*, 1909, 154.

F. Hughes and H. C. Jefferys, "Manurial trials on Cotton carried out in the Stata Domains, 1910," *Agric. Journal Egypt*, 1912, **1**, 8.

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The important work of W. L. Balls on the physiology of the Egyptian cotton plant has thrown considerable light on the factors limiting the growth of this crop, and undoubtedly other factors, such as water supply and temperature, limit the growth and ultimate yield of the cotton crop far more than does the food supply.

As an outcome of the results obtained by the study of the soil of cotton fields during the past two years and described in the following pages, the writer has a suggestion to make concerning this problem of the nutrition of the Egyptian cotton plant, at least as far as the nitrogen is concerned.

The cereals of Egypt, however, are in a very different position. To persons accustomed to hear of the inexhaustible fertility of the Nile valley it comes somewhat as a shock to learn how dependent the maize and wheat crops are on nitrogenous fertilisers. The rapid increase in the sales of nitrate of soda during the past ten years and the exploitation of all local sources of nitrogen, such as the débris of ancient villages (Koufri), are signs that the fertility of Egyptian soils is indeed not permanent under the modern system of perennial irrigation. The lack of fuel is so pronounced in Egypt that all crop residues are gleaned from the land for this purpose and all solid excrements of farm animals are converted into fuel. At the time of writing, cotton seed cake, containing 4 % of nitrogen, is one of the most important commercial fuels of the country. The use of green manures, in the real sense of the term, is quite unknown in Egypt.

The value of the leguminous crop, Bersim, in maintaining the fertility of the soil is well known by the farmers themselves and when fed on the land a considerable proportion of nitrogen is added to the soil. Under these conditions the amount of nitrogen added to the soil by the bersim crop ought to be sufficient to carry forward the rotation of three years, with a little artificial aid to the maize crops.

The residues of the bersim alone without any return to the land in the way of animal excrements would seem however to be insufficient for the successful growing of cereal crops.

NITRIFICATION IN EGYPTIAN SOILS.

Up to the present time no definite series of observations has been made in Egypt concerning the activity of soil bacteria in producing plant food. Beyond the general statement by Roche, already mentioned, that nitrification does take place, no evidence for example is available concerning the intensity of the process as determined by the fluctuation

of the nitrate content of the soil. In the following study, the amount of nitrate present in the soil has been taken as an index of bacteriological activity. This method has found successful application in other parts of the world and without doubt is one of the simplest and best. More recently, the determinations of soil nitrates have been supplemented by bacterial counts and by observations on the atmosphere surrounding the soil particles[1].

Under the conditions of the writer's laboratory, it has been difficult to supplement the nitrate values except in some special cases.

The amount of nitrate found in the soil at any moment is usually the result of the balance between crop requirements, drainage and nitrification. In Egypt there is little or no drainage under normal conditions, on the other hand other movements of soil water are of importance, such as the vertical movements due to capillarity and lateral movements due to seepage. In the first case salts accumulate on the higher portions of the soil such as the tops of ridges, while in the latter case considerable areas at lower levels are affected. In some cases nitrates in considerable quantities may be found out of the reach of plant roots on the tops of ridges which only receive water from below by capillarity. A case of this kind is quoted in connection with a maize field[2].

The case of fallow land with its accumulation of nitrates so well known in England is of special interest in Egypt. Fallow soils in Egypt may be and frequently are biologically dormant; in some cases, as in the basin lands of Upper Egypt, for several months each year. The water supply has been cut off and moisture conditions are therefore unsuitable. The fallow periods of the Egyptian farm rotation are particularly interesting on this account.

When a crop is grown on the land nitrate is removed from the soil fairly rapidly. In the case of wheat and maize there is usually no accumulation of nitrate in the soil, but the case of cotton is remarkable in that apparently, in the early stages of growth at least, nitrification is well ahead of the needs of the plant and appreciable quantities of nitrate accumulate in the surface soil.

[1] See for example: E. J. Russell, This *Journal*, 1914, **6**, 18; E. J. Russell and A. Appleyard, This *Journal*, 1915, **7**, 1; E. J. Russell and A. Appleyard, This *Journal*, 1917, **8**, 385.

[2] See also V. M. Mosseri, "Note préliminaire sur les sels nuisibles et le cotonnier en Egypte," *Trans. 3rd International Congress of Tropical Agriculture*, London, 1914.

Land in preparation for cotton.

There are two possibilities in the preparation of land for the cotton crop; either the land remains fallow after the preceding crop of maize or more usually a catch crop of bersim is grown, which enriches the soil somewhat but does not always permit of the thorough soil cultivation which the growing of cotton demands.

The winter fallow.

In the winter fallows of 1916–1917 and of 1917–1918 the progress of nitrification in the land in preparation for cotton was followed up. The preceding crop in each case was maize, which usually leaves little nitrate in the soil. In the first winter, the maize crop was harvested in November, 1916, and the land was first ploughed on December 4th. Repeated cultivations were given during the winter in preparation for the succeeding cotton crop. The rainfall may be taken at 40 to 50 mm. The moisture content of the soil remained remarkably uniform at 18 to 20 %, conditions which afforded a good opportunity for nitrification. Soil temperatures were about 16° C. Throughout the whole period there was a steady accumulation of nitrates in the surface soil from 9 parts to 23 parts per million of dry soil. After the final ridging for cotton on

Table III.

Nitrates in fallow land in preparation for Cotton. 1916–1917.

Date	Temperature at the time of sampling	Nitric nitrogen. Parts per million of dry soil	Moisture %
1916			
Nov. 13	—	9·0	19·2
Dec. 14	—	12·9	18·2
1917			
Jan. 15	15·2° C.	19·2	18·6
Feb. 14	15·0° C.	23·0	19·7
Mar. 6	17·0° C.	21·0*	16·2
Mar. 22	19·9° C.	35·5*	20·0

Agricultural operations.

1916.	Nov. 5	Maize harvested.
	Dec. 4	Ploughing.
	Dec. 27	Ploughing.
1917.	Feb. 15	Ridging.
	Mar. 6	Seed sowing.
	Mar. 8	Irrigation.

* In the subsoil (25–50 cm.) on Mar. 6 and Mar. 22, 14 parts per million of nitric nitrogen.

February 15th and up to March 6th there was no further increase in the nitrate content of the soil probably on account of the relative dryness. Immediately after the cotton was sown and watered the conditions became favourable and in sixteen days the nitrates increased from 21 to 36 parts per million. Table III gives the results of the 1916 1917 observations.

In the second series, carried out in 1917 1918, the maize was harvested at the end of October and 13 parts per million of nitrate nitrogen were present in the surface soil. In this case, however, the accumulation of nitrate during the winter was negligible. The soil was poor and the rainfall was less than in the preceding winter, so that there were few favourable opportunities when the moisture content of the soil was sufficiently high for active nitrification. As with the field of 1917, as soon as irrigation began, nitrification increased and an accumulation from 14 to 28 parts per million was observed in 20 days. In Table IV the results of the observations of the winter 1917 1918 are given.

TABLE IV.

Nitrates in fallow land in preparation for cotton. 1917-1918.

Date	Temperature at the time of sampling	Nitric nitrogen. Parts per million of dry soil	Moisture %
1917			
Oct. 31	22·0° C.	12·8	18·0
Dec. 19	14·9° C.	14·1	18·9
1918			
Jan. 28	14·0° C.	10·4	19·2
Feb. 28	16·3° C.	13·8	18·8
Mar. 23	19·0° C.	27·6	21·3

Agricultural operations.

Maize harvested	October 28, 1917.
Ploughings	Nov. 8.
	Jan. 4, 1918.
	Feb. 12.
Ridging	Feb. 23.
Seed sowing	Mar. 2.
Irrigation	Mar. 4.

The difference between the results obtained in the two winters is most probably explained by the difference between the rainfalls. In the following Table V the official records of the Egyptian Ministry of Public Works for the two stations nearest to Bahtim are given.

Table V.

Rainfall near Cairo, 1916–1917, *mm.*

	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Total
Abbassia	0·0	2·5	1·5	29·8	13·7	1·5	49·0
Giza	0·0	3·8	1·6	17·3	9·0	0·0	31·7

Rainfall near Cairo, 1917–1918, *mm.*

	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Total
Abbassia	0·0	7·5	3·2	0·5	4·4	15·0	30·6
Giza	0·0	1·4	3·1	1·1	2·3	12·0	19·9

In the first winter the rainfall was not only higher than in the second winter but was distributed as a small number of fairly heavy showers. At Abbassia 25 mm. fell on January 2, 3 and 4, 1917, and 7·0 mm. on February 10 and 11. In 1917–1918 more frequent, but very light rains were the rule; in only one or two cases did more than one millimetre fall in one day. Further in 1918 the bulk of the rain fell in March when the cotton was already growing on the land under observation. In 1917 the wettest month was January.

It is possible that the nitrification is not continuous, but occurs only after showers heavy enough to supply the necessary soil moisture. This would explain the differences observed between the two winters.

The bersim catch crop.

The growing of a single cutting of bersim during the winter preceding the cotton crop is very common as the extra fodder so obtained is very valuable. The amount of nitrogen returned to the soil in the excrements of the animals feeding on this crop together with the crop residues has been estimated by the writer to be not more than 55 to 65 pounds per

Table VI.

Nitrates in fallow soil after bersim catch crop. 1918.

Date	Soil Temperature	Nitric nitrogen. Parts per million of dry soil		Moisture %	
Jan. 9	12·4° C.	13·7		18·9	
Feb. 28	14·5° C.	14·7		16·4	
Mar. 23	18·9° C.	19·0		22·2	
May 8	25·0° C.	33·4	48·3	15·0	15·3*
May 28	25·0° C.	28·5*	19·7	21·3	19·5
July 14	31·1° C.	28·3	25·3	6·5	10·7

* The subsoil contained nitrates: 13·7 parts per million. Irrigations were given on March 4 and May 16.

acre, that is, just about sufficient for the requirements of the cotton crop. Owing to the long growing season of the cotton crop and the active nitrification going on all the time, probably very little of this nitrogen is available for the crops which follow the cotton. The nitrification of the residues from the bersim catch crop was followed up in two cases, the land was irrigated occasionally and hoed to keep down the weeds, but was otherwise not treated.

The above results illustrate the necessity for water in keeping up the biological activity in these soils. The loss in nitrate indicated above may possibly be accounted for by denitrification, but on the other hand the results are very similar to those discussed by E. J. Russell and A. Appleyard in their second paper[1].

Nitrification under the cotton crop.

Observations extending over two years have been made on the fluctuations in the nitrate content of the surface soil of a cotton field. In 1917 a field of cotton was chosen under normal farming conditions, following the winter fallow which had been studied in the preceding winter. In 1918, a plot of cotton was chosen, the soil of which had received no manure for some years and which, judging by the yield of cereals, was in a fairly exhausted condition. The fallow period of this plot was that studied in 1917–1918.

In 1918 also, the early period of an experiment on the sowing date of the cotton crop afforded a suitable opportunity for the study of biological activity in the soil as affected by the different dates for the first irrigation.

The cotton field of 1917.

The results obtained during 1917 are given in the following Table VII. Determinations of soil nitrates were made as far as possible between each irrigation period.

The main feature of the conditions observed in 1917 is the relatively high amount of nitrate present in the surface soil of the cotton field at all stages of growth. The effect of irrigation in stimulating nitrification is shown in the observations made about the irrigation of June 21.

[1] This *Journal*, **8**, 403.

Table VII.

Amounts of nitrate found in the surface soil of a cotton field. 1917.

Date	Temperature at the time of sampling	Nitric nitrogen. Parts per million of dry soil	Moisture %
Mar. 6	17·0° C.	21·0	16·2
Mar. 22	19·9° C.	35·5	20·0
Apr. 30	29·0° C.	28·3	19·9
May 26	26·5° C.	22·5	16·8
Jun. 17	31·4° C.	16·8	15·6
Jun. 22	—	26·2	28·4
Jun. 26	27·6° C.	22·7	22·4
Jul. 10	28·0° C.	30·2	14·3
Aug. 1	27·0° C.	33·3	16·5
Sep. 4	25·5° C.	21·4	21·0
Sep. 15	24·8° C.	18·1	16·7
Oct. 24	20·5° C.	15·8	17·3

Agricultural operations.

March 6. Seed sowing.
March 8. Irrigation.

Other irrigations: March 22, April 28, May 25, June 21, July 12, July 31, August 19, October 2.

October 28. Plants removed from the ground.

The cotton field of 1918.

The characteristics of the observations made in 1918 are quite similar to those observed in 1917. The results are given in Table VIII.

Table VIII.

Amounts of nitrate found in the surface soil of a cotton field. 1918.

Date	Temperature at the time of sampling	Nitric nitrogen. Parts per million of dry soil	Moisture %
Feb. 28	16·3° C.	13·8	18·8
Mar. 23	19·0° C.	27·6	21·3
April 13	23·7° C.	16·8	18·9
April 17	22·1° C.	33·9	22·5
June 2	27·5° C.	60·0	16·3
June 25	27·6° C.	32·7	16·4
July 3	26·0° C.	33·5	13·5
July 20	29·9° C.	25·1	13·5
Aug. 28	33·2° C.	16·6	12·0
Sept. 10	26·4° C.	26·4	22·1

Agricultural operations.

Seed sowing: March 5.

Irrigations: March 5, April 13, May 23, June 16, July 4, July 22, August 6, September 5, October 5.

In 1918, a further study was made on a piece of land, which was under experiment for the sowing date of cotton. The land was rather rich and nitrification was followed up on the soil of plots sown on the 1st February, 1st March and the 1st April. The land was too dry before the irrigation of the cotton crop to allow of any bacteriological activity and it was possible to compare land under cotton with the corresponding fallow piece waiting for irrigation after a month's interval. Considerable quantities of nitrate were observed, up to 60 parts per million. The results indicate the rapid accumulation of nitrate that is possible in an Egyptian cotton field far in excess of the immediate requirements of the plant. See Table IX.

TABLE IX.

Amounts of nitrate found in the surface soil of cotton plots sown on different dates. 1918.

Date	Temperature of soil at the time of sampling	Nitric nitrogen. Parts per million of dry soil	Moisture %	Sowing date of cotton
Jan. 31	14·0° C.	25·1	17·0	
Feb. 16	15·9° C.	31·6	18·9	
Mar. 19	17·9° C.	42·7	20·4	Feb. 1
Apr. 16	23·4° C.	52·9	20·3	
May 16	27·0° C.	37·1	11·3	
June 25	27·7° C.	39·7	14·3	
Feb. 28	16·1° C.	17·0	17·8	
Mar. 19	17·9° C.	42·3	22·1	
April 16	23·4° C.	38·7	19·2	Mar. 1
May 16	27·0° C.	42·8	11·5	
June 25	27·7° C.	48·7	14·8	
April 1	22·8° C.	18·0	12·8	
April 16	23·4° C.	42·9	21·2	
May 16	27·0° C.	60·6	10·2	April 1
June 25	27·7° C.	48·0	14·1	

Irrigations—February plot: February 1, March 1, April 11, May 23, June 14.
March plot: March 1, April 11, May 23, June 14.
April plot: April 1, May 23, June 14.

The amount of nitrogen required by the cotton crop is stated by Foaden and Mackenzie[1] to be 59 pounds per acre. The writer has made two rough determinations of the nitrogen required at early stages of growth. The amount of nitrogen required in the first two or three months is more than supplied by nitrates produced in the surface soil.

[1] W. C. Mackenzie and G. P. Foaden, *Manures in Egypt and Soil Exhaustion*, Cairo, 1896.

In fact, quantities of nitrate nitrogen have been found at Bahtim in the soil of cotton fields quite sufficient for the growth of the crop to the end of the season. The cotton crop in Egypt is not therefore usually limited by the food supply. Table X illustrates approximately the relationship between the requirements of the cotton crop and the amount of nitrate formed in the soil by biological processes.

TABLE X.

Nitrogen intake of the cotton crop and nitrate production in a cotton field. Pounds per acre.

	May	June	July	Aug.	Sept.
Nitrogen required by cotton crop	4	—	40	—	60
Nitrates produced in surface soil	80	120	140	—	—
Root depth according to W. L. Balls[1]	50 cm.	90 cm.	150 cm.	180 cm.	200 cm.

The distribution of nitrate between soil and subsoil has not been followed up as yet. The movements of the salts in the soil with each irrigation would afford some clue as to what happens to the nitrates after they are produced. The only results obtained so far indicate, as would be expected, that the amount of salt in the soil bears no relationship to the amount of nitrate present. In each case the quantity present is the outcome of dissimilar factors. The results of W. L. Balls show that irrigation affects the moisture content of the soil down to considerable depths, so that there is no reason to suppose that the nitrate accumulated in the surface layer is never washed down far enough to feed the plant.

Table XI illustrates the lack of relationship between the amounts of salt and of nitrate in the soil of the cotton field of 1917.

TABLE XI.

Amounts of nitrate and of sodium chloride in the surface soil of a cotton field. 1917.

Date	Moisture %	Nitric nitrogen. Parts per million of dry soil	Sodium Chloride %
June 22	28·4	26·2	0·015
June 26	22·4	22·7	0·004
Sept. 15	16·7	18·1	0·010
Oct. 24	17·3	15·8	0·008

[1] This *Journal*, 1913, 5, 469.

Nitrification in a maize field.

The Egyptian maize crop grows exceedingly rapidly—in some sixty to eighty days the whole of the nitrogen required by the crop has been taken up and in consequence nitrification in the soil has to be very active to keep up with the requirements of the plants. Maize practically always follows the summer fallow or "sheraqi" and the period of ten days between the irrigation of the sheraqi and the sowing of the crop does not allow of much nitrate accumulation. Two cases were observed in 1917, one plot unmanured after wheat, and a second plot unmanured after bersim. After the first irrigation the amount of nitrate in the soil increased from 6 parts to 13 parts per million in nine days; during the period of growth the amount of nitrate in the land after bersim rose at first to 15 parts and then fell gradually to 5 parts per million as the maize took up increasing quantities of nitrogen, later rising again to 15 parts per million during the period of maturation of the crop. The corresponding plot after wheat shows a more steady content of nitrate, about 10 parts per million of dry soil. In neither case is there any marked accumulation of nitrate. The higher values obtained in the earlier and later stages after bersim reflect the higher nitrogen content of the soil after a leguminous crop. See Table XII.

TABLE XII.

Nitrates found in the surface soil of maize plots. 1917.

		After bersim		After wheat	
Date	Temperature at the time of sampling	Nitric nitrogen. Parts per million of dry soil	Moisture %	Nitric nitrogen. Parts per million of dry soil	Moisture %
June 26	—	6·1	6·9	5·4	5·8
July 5	25·5° C.	12·0	23·5	12·9	22·8
July 25	31·6° C.	15·3	18·5	10·6	19·6
Aug. 16	30·6° C.	8·1	13·7	9·9	15·5
Sept. 15	25·0° C.	5·4	21·4	11·1	20·1
Oct. 31	22·0° C.	14·8	21·7	12·8	18·0

Agricultural operations.

June 28. First irrigation of sheraqi.
July 7. Ploughing and seed sowing.
July 30, August 17, Sept. 20, Oct. 6. Irrigations.
Oct. 28. Maize harvested.

The amount of nitrate found in the surface soil of a maize field is frequently the outcome of factors other than those usually considered in the case of similar studies made under English conditions. Maize is

a plant needing a considerable amount of space, and at the present time in Egypt there is considerable discussion concerning the spacing to be adopted and the special cultivation to be given. In a manuring experiment conducted at Bahtim in 1917 a system of cultivation on ridges was adopted which later experience showed to have been inadvisable. A number of nitrate determinations was made in this field and the relatively high values obtained, particularly at a time when the plant was most active in taking up nitrate, would indicate that the method of planting adopted resulted in a waste of nitrate as far as the maize crop was concerned. Some of the results are given in the following Table XIII. Apparently both the nitrate added to the soil in the fertiliser and that produced in the soil by biological activity is to an appreciable extent dispersed in the soil out of the reach of the roots of the maize plants.

TABLE XIII.

Amounts of nitrate found in the soil of a maize field. Cultivation on ridges. 1917.

Date	Nitric nitrogen. Parts per million of dry soil		Moisture %		Manuring (lbs per acre)
	Soil	Subsoil	Soil	Subsoil	
Oct. 1	16·7	11·4	19·4	17·0	Unmanured
	14·7	11·8	19·3	18·3	Nitrate of soda (333) applied in two dressings to crop
	19·4	14·4	19·2	19·5	Nitrate of soda (333) applied in one dressing before sowing
	17·9	18·0	18·5	18·7	Sulphate of ammonia (253) applied in one dressing before sowing

A further series of determinations was made at the end of the season in the case of an experiment on the spacing of the maize crop. The results are given in Table XIV.

TABLE XIV.

Amounts of nitrate and salts in the surface soil of maize plots. Bahtim spacing experiments. 1917. *End of season, Dec.* 22.

Method of planting	Nitric nitrogen. Parts per million of dry soil	Sodium Chloride %	Total Salts %
Ridges. Thick sowing ...	31·5	0·013	0·104
Ridges. Thin sowing ...	42·3	0·012	0·108
Flat. Thick sowing ...	20·4	0·016	0·100
Flat. Thin sowing ...	17·8	0·013	0·104
Tops of ridges. Thick sowing ...	601·0	0·074	0·652
Tops of ridges. Thin sowing ...	647·7	0·103	0·780

The above results indicate very clearly that the sowing of maize on ridges results in the waste of a considerable quantity of nitrate. The very high values obtained for the tops of the ridges show the effect of capillarity in the accumulation of soluble salts in the surface soil under arid conditions of agriculture. The high value for the sodium chloride and for the total salts is also an index of this factor.

As would be expected from the above considerations, the maize crop in Egypt responds readily to nitrogenous manuring, particularly to nitrate of soda.

The question of the relationship between the cultivation and manuring of the maize crop is illustrated by two series of experiments conducted at Bahtim during 1917 and 1918. In 1917 each treatment was repeated on five plots and the maize was grown on ridges; the results in Table XIII relate to this series. In 1918, each treatment was repeated on four plots and the maize was grown on the flat at two different spacings. The waste of nitrate on the ridges indicated in Table XIII is brought out in the yields obtained at the end of the season.

TABLE XV.

Yields of maize. Bushels per acre.

1917 Crop: cultivation on ridges	
Unmanured	31·1 ± 1·4
Nitrate of soda (333 lbs) applied before sowing	43·5 ± 0·9
Nitrate of soda (333 lbs) applied half before sowing and half at first irrigation	47·7 ± 2·1
Nitrate of soda (333 lbs) applied half at the first irrigation and half at the second	50·8 ± 3·1
Nitrate of soda applied at the first irrigation	49·2 ± 1·9
Ammonium sulphate (equivalent) applied before sowing	39·0 ± 1·1
1918 Crop: cultivation on the flat	
Unmanured	37·0 ± 1·9
Nitrate of soda (333 lbs), half at first irrigation and half at second irrigation. Close planting	62·8 ± 0·8
Nitrate of soda (333 lbs) applied before sowing. Close planting ...	60·2 ± 1·2
Ammonium sulphate (equivalent) before sowing. Close planting ...	61·2 ± 0·8
Nitrate of soda (333 lbs), half at first irrigation and half at second irrigation. Wide planting	58·8 ± 0·7
Nitrate of soda (333 lbs) applied before sowing. Wide planting ...	58·9 ± 0·5
Ammonium sulphate (equivalent) before sowing. Wide planting ...	59·8 ± 0·7

Close planting at 35 cm. Wide planting at 55 cm.

Nitrification under the wheat crop.

Wheat in Egypt usually follows the cotton or the maize crop, and on weak land responds to nitrogenous manures but not so readily as does maize. The few results tabulated below show that as in England nitrates do not accumulate under the wheat crop. The amounts of nitrate found in the stubbles of the wheat in the case of the summer fallow is a further illustration of this point.

TABLE XVI.

Amounts of nitrate found in the soil of wheat field. 1917-1918.

Date	Temperature at the time of sampling	Nitric nitrogen. Parts per million of dry soil	Moisture %
1917			
Dec. 19	14·9° C.	15·5	20·2
1918			
Jan. 28	14·0° C.	10·4	19·2
Mar. 23	18·7° C.	7·4	24·0
June 2	27·5° C.	5·5	7·4

The summer fallow ("sheraqi").

One of the most characteristic periods of the Egyptian farm rotation is the period following the winter crops. The land is already in a fairly dry condition when the crop is removed and it is allowed to remain fallow without treatment of any kind until sufficient water is available for the "Nili" crop. The earliest date at which the first irrigation of the sheraqi is allowed is fixed by law and depends on the prospects of the Nile flood. A very intensified form of sheraqi is observed in Upper Egypt on the basin lands which only receive water at each Nile flood and are fallow from May until August. A number of nitrate determinations was made on sheraqi soils immediately after the removal of the winter crops in some cases, and in others after an interval or just before the irrigation. The moisture after the removal of the crop may be fairly high according to the previous irrigation of the field, but the soil soon dries out and the moisture content falls to 6 or 7 %, or a little below the air dried condition that is reached under laboratory conditions. The preceding crop has removed most of the soluble nitrogen from the soil. Even after bersim little nitrate is found unless stock have just been feeding on the land. See Table XVII.

TABLE XVII.

Moisture and nitrate content of "sheraqi" soils.

Date	Preceding crop	Temperature at the time of sampling	Nitric nitrogen. Parts per million of dry soil	Moisture %
1917				
May 3	Flax*	21·0° C.	2·7	14·2
May 3	Flax†	—	2·7	15·5
May 26	Wheat	31·0° C.	4·7	15·4
May 30	Wheat‡	—	7·3	7·1
	Wheat‡	—	8·0	7·4
	Wheat§	—	15·4	7·7
	Wheat§	—	11·7	8·3
June 26	Wheat	31·0° C.	5·4	5·8
June 26	Bersim	—	6·1	6·9
1918				
June 2	Wheat	27·5° C.	4·3	8·5
Aug. 8	Basin Assiut	36·0° C.	6·8	3·8
	"	—	5·7	3·7

* Flax manured with farm manure. † Flax manured with nitrate of soda.
‡ Wheat: unmanured plots. § Wheat: nitrate plots.

The general conditions of the sheraqi include fairly high temperatures. At Bahtim in June, 1917, just before the irrigation the highest temperature observed was 35° C. at 10 cm. below the surface. At Assiut in August, 1918, on a basin 46° C. was observed but undoubtedly higher temperatures are the rule during the months of June and July. As might be expected no bacteriological activity can take place under such conditions and nitrates do not accumulate in sheraqi soils. The number of bacteria is also low, usually not more than one million per gramme of dry soil. See Table XVIII.

TABLE XVIII.

Bacteria present in sheraqi soils. Millions per gramme of dry soil.

	Bacteria	Moisture %
Wheat soil. Bahtim, 1917 ...	2·0	7·0
Bersim soil. Bahtim, 1918 ...	1·6	4·1
Basin soil, Assiut, 1918. Hod el Zenar	1·1	3·8
Basin soil, Assiut, 1918. Hod el Zenar	1·0	3·7
Basin soil, Shotb village, 1918...	0·8	5·3

The normal number of bacteria in the soils of Egyptian fields appears to be about 10 to 15 millions per gramme of soil.

It might be expected that as soon as conditions again became favourable for biological activity, an abnormal increase in such processes as nitrification would take place. E. J. Russell and H. B. Hutchinson[1] suggest that prolonged drought or prolonged heating at 40° C. acts on the soil in the same way as partial sterilisation by heat or by volatile antiseptics. No doubt in the case of the basin lands some such action does occur and to a less extent in the ordinary sheraqi soils of Lower Egypt. The conditions are not quite stringent enough in the case of the latter soils to kill off all the protozoa which are readily found in sterile hay infusion which has been inoculated with sheraqi soil. In the case of the basin samples however, very few protozoa developed, chiefly ciliates, and these were by no means vigorous. The proof of the partial sterilising effect of the sheraqi conditions is rather difficult to obtain, as it is obviously difficult to keep the same soil untreated for comparison. The writer has made preliminary attempts to settle this interesting point, but further study will be necessary before final evidence can be obtained.

The summer fallow subsequent to the sheraqi period.

Frequently after a bersim crop, the summer fallow is extended beyond the sheraqi period until the winter, when a crop of wheat is grown. The irrigation necessary is given after the requirements of the maize crops have been satisfied. During the summer of 1917, the amount of nitrate accumulated in such soils was determined in a few instances. A piece of land after wheat, reserved for such a fallow, showed an increase in nitrate content from 14 to 23 parts per million in one month. Another piece of land after bersim contained 26 parts per million on the 3rd November after being fallow all the summer; in this case no special effort had been made to keep the soil moist after the first irrigation and ploughing. The possibilities of the active summer fallow were illustrated, however, in the case of two fallow pots containing forty kilos each of soil, which were kept at 20 % moisture content for three months during the summer of 1917. The amount of nitrate in the soil increased from 10 parts to 250 parts per million during this period. Under practical conditions it would hardly be possible to obtain such active nitrification in the summer fallow—and possibly not advisable to attempt it. All that is necessary is to obtain a sufficient accumulation of nitrate in the soil to give the wheat crop a good start, relying on the

[1] This *Journal*, 1913, 5, 152.

nitrification under the wheat itself for the ultimate requirements of the crop.

Effect of a growing crop on nitrification.

It has been suggested that the growing crop influences the biological activity of the soil. The general opinion seems to be that nitrification in a cropped soil is not so active as in the corresponding fallow soil. In the case of the maize crop, however, T. L. Lyon and J. A. Bizell[1] suggest that the effect of the crop is to stimulate nitrification. In the case of Egyptian soils it is almost impossible to compare cropped soils with fallow soils under field conditions owing to the enormous variation in the water content of these soils. A number of data from pot experiments are available, however, and are of sufficient interest to be included in this paper.

TABLE XIX.

Effect of maize crop on nitrification in the soil.
Pot experiment. 1917.

Date	Soil moisture %	Nitrogen in crop gm.	Nitric nitrogen found in pot gm.	Total nitric nitrogen produced gm.
Aug. 2	7	0	0·39	0·39
Sept. 11	22	0·68	1·40	2·08
Oct. 2	19	1·60	0·63	2·23
Nov. 7	24	1·30	0·79	2·09
Nov. 18	20	1·54	0·72	2·26
Nov. 18	20	Fallow pot	5·76	5·76

TABLE XX.

Effect of a wheat crop on nitrification in the soil.
Pot experiment. 1917-1918.

Days	Soil Moisture %	Nitrogen in crop gm.	Nitric nitrogen found in pot gm.	Total nitric nitrogen produced gm.
0	—	0	1·38	1·38
78	14	0·73	0·94	1·68
112	15	1·37	0·70	2·07
176	18	1·34	0·61	2·04
176	—	Fallow pot	3·68	3·68
0	—	Fallow pot	1·38	—
23	—	—	1·87	—
71	—	—	2·61	—
129	—	—	3·68	—

[1] *Journ. Franklin Inst.*, 1911, Jan.

These results would indicate that the root activities of a growing crop have some limiting effect on the production of nitrate in the soil. In both cases the fallow pots have accumulated appreciably more nitrate than have the cropped pots. The difference between the results obtained with maize by Lyon and Bizell and those reported above is probably to be explained by the fact that in the United States the maize crop is given much more space than is usually the case in Egypt. The maize plants in the pots were certainly overcrowded.

Sampling and determinations.

The plots to be investigated were sampled with a soil borer to a depth of 25 cm. in at least four places and the sample brought to laboratory immediately. Duplicate lots of 250 gm. each were dried at 55° C. as in the Rothamsted method and the nitrates washed out on a Buchner funnel with distilled water. The reduction to ammonia was carried out in alkaline solution with Devarda alloy or preferably by means of a mixture of zinc dust and reduced iron.

CONCLUSIONS AND SUMMARY.

An attempt has been made to determine the intensity of the biological processes in the soil during the most important periods of the Egyptian farm rotation. The fluctuations of the nitrate content in the surface soil have been taken as the most important index of this activity.

In all cases the moisture content of the soil limited these processes more than any other factor.

There was observed throughout the season in a cotton field a relatively large amount of nitrate, more than sufficient for the immediate needs of the cotton plant. The lack of response on the part of the Egyptian cotton crop to nitrogenous fertilisers may be accounted for in part, if not entirely, by the fact that nitrification in the soil is well ahead of the needs of the crop.

Nitrification under wheat and maize shows in general the same characteristics in Egypt as in other parts of the world; there is no accumulation of nitrate in the soil.

The winter fallow, depending for its water on the rainfall, may be a period of steady nitrification when the amount of the rainfall is sufficiently high.

The "sheraqi" soils of the summer fallow are biologically dormant. They are characterised by very low moisture content and by fairly high temperatures, particularly in the basins of Upper Egypt. There is a probability that these conditions act as a partial sterilisation of the soil.

Pot experiments are described concerning the effect of the growth of maize and of wheat on the accumulation of nitrate in the soil.

BAHTIM EXPERIMENTAL STATION,
March, 1919.

(*Received May* 10*th*, 1919.)

THE DISTRIBUTION OF DRY MATTER AND NITROGEN IN THE POTATO TUBER. VARIETY, KING EDWARD.

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The following investigations arose out of certain work undertaken at the Rothamsted Experiment Station in 1918 in connection with the Food Production Department. This involved sampling a large number of tubers. Investigations into the best method of sampling suggested the advisability of studying in detail the distribution of dry matter and nitrogen in the different parts of the tuber.

PREVIOUS INVESTIGATIONS.

As early as 1892 M. Douillet(1) discussed the uneven distribution of starch in the different parts of the tuber. He showed that chemical analysis and microscopical examination reveal the fact that starch is much more abundant near the periphery than in the inner part of the potato, and that the grains appear to be formed in the neighbourhood of the vascular system. He also showed that the two ends of the tuber vary in starch content but not always in the same direction. At the time of lifting the starch content is higher towards the point of attachment than towards the sprout end, but the reverse is true when the buds are sprouting.

M. Douillet used the coring method for sampling, but this was later criticised by Coudon and Bussard who point out the difficulty of directing a core exactly along an axis. Slight deviation from the axis might cause serious error.

Doerstling(2) in 1895 arrived, by a completely different method, at conclusions which agree in the main with those of later workers. He took a core along one of the axes of the tuber, divided it into discs 2 mm. thick and determined the specific gravity of each disc. He argued from

his results that the parts of highest specific gravity are all in one zone which is nearer the periphery than the central part of the tuber, and further from the end of attachment than from the sprout end. The criticism of the coring method again applies to this work.

Coudon and Bussard (3), two French investigators, gave in 1897 the percentage dry matter and nitrogen in different zones of the tuber. In zoning they followed the main physical divisions which can be seen with the naked eye. The skin forms a corky covering surrounding the cortical layer. The latter is generally the densest part of the tuber, and its inner boundary is roughly marked by an incomplete ring of vascular bundles whose branches ramify in the cortical and in the central zone. The central or medullary area varies considerably in uniformity. The interior part is very wet and translucent, and has branches known as pith rays which penetrate the denser outer medullary region and sometimes reach the surface of the tuber in the neighbourhood of the eyes.

The zones taken by Coudon and Bussard are as follows:

Skin.
Cortical Zone.
External Medullary Zone.
Internal ,, ,,

They express their results for nitrogen as percentage protein in fresh material, *i.e.* total nitrogen × 6·25. For purposes of comparison we have calculated the percentage of nitrogen in both fresh and dry material. The results are shown in Table I.

TABLE I. *Composition of the Different Zones of the Potato.*

Coudon and Bussard.

Variety	Zone	% of whole tuber	% dry matter	% nitrogen in fresh material	% nitrogen in dry matter
Bleue gearite	Skin	9·57	17·16	0·364	2·120
	Cortical	34·66	27·53	0·306	1·110
	External medullary	37·15	25·67	0·299	1·166
	Internal ,,	18·62	18·28	0·342	1·868
Czarine	Skin	8·02	17·79	0·360	2·022
	Cortical	37·72	27·08	0·295	1·089
	External medullary	38·11	21·13	0·347	1·640
	Internal ,,	16·15	15·52	0·348	2·241

The percentage of dry matter is less in the skin than in the cortical layer and decreases from the cortical to the internal medullary layer. The percentage of nitrogen is greatest in the skin and is lower in the

cortical than in the medullary area. The percentage of nitrogen in the fresh material does not vary greatly in the different zones while the dry matter varies much. It follows that the percentage nitrogen in the dry material varies considerably in the opposite direction to the dry matter.

Again Coudon and Bussard divided the tuber by two cuts at right angles to the long axis, into an umbilical, an intermediate and a terminal region. Each of these they divided into zones as before. They found that the general relation of the cortical, the outer and inner medullary zones to each other is the same in each of the three parts. The cortical zone is always drier and poorer in nitrogen than the internal medullary, and the external medullary is intermediate between the two.

Shortly after the appearance of this work, Frisby and Bryant(4) published analyses of different zones of the American variety 'White Star.' Their method of zoning differed from that of Coudon and Bussard. They scraped off both the dry brown outer covering which they termed the "outer skin" and the layer immediately below this, the "inner skin." The latter contains whatever colouring matter is present in the tuber. The remaining flesh was treated as a whole. Their results are shown in Table II.

TABLE II. *Composition of Different Zones of the Potato Tuber.*

Frisby and Bryant.

Variety	Zone	% of whole tuber	% dry matter	% nitrogen in fresh material	% nitrogen in dry material
White Star	Skin, outer	2·5	19·9	0·43	2·16
	" inner	8·5	16·8	0·36	2·14
	Flesh	89·0	18·9	0·32	1·69
	Skin, outer and inner	11·0	17·5	0·38	2·14

The inner and outer skin taken together constitute 11 per cent. of the whole tuber, while the skin or "enveloppe" of two varieties analysed by Coudon and Bussard form respectively 8·02 and 9·57 per cent. of the whole. A certain variation with the size, shape and variety of the tubers is inevitable. The figures quoted therefore approximate sufficiently to support the supposition that the two skins referred to by Frisby and Bryant correspond roughly to the whole "enveloppe" or skin of Coudon and Bussard. Further Coudon and Bussard admit that part of the subcutaneous layer always adhered to the skin when it was removed. This is very difficult to avoid, but Frisby and Bryant took particular precautions against it.

Unfortunately some confusion has arisen from Frisby and Bryant's suggestion of the terms fibro-vascular or cortical layer for the inner skin.

This misled East into assuming a correspondence between the cortical layer of Coudon and Bussard and the inner skin of Frisby and Bryant. The former constitutes about 35 per cent. of the whole and the latter only 8·5 per cent.

Frisby and Bryant throw light on the somewhat surprising result of Coudon and Bussard's work, that the apparently dry skin contains less dry matter than the obviously wet flesh of the tuber. It appears that there is a very wet layer, the inner skin immediately below the dry outer cuticle. This, which wholly or partially is generally removed with the outer skin, gives the low figure for its percentage dry matter. When taken together inner and outer skin contain a smaller proportion of dry matter than the flesh while each layer contains a higher percentage of nitrogen. These results agree with those obtained by Coudon and Bussard.

Further investigations were published by Waterstradt and Willner (5) in 1901. They divided the tuber into cortical and medullary areas, but did not separate the external and internal medullary. The average results for three varieties of good table quality and for three coarse starch producing varieties, both types grown on two separate fields, are shown in Table III.

TABLE III. *Composition of Zones in the Potato.*
Waterstradt and Willner.

Variety	Zone	% dry matter	% nitrogen in fresh material	% nitrogen in dry matter
Eating varieties ...	Cortical ...	24·3	0·336	1·34
Berlin Exp. Field ...	Medullary ...	19·8	0·363	1·85
Eating varieties ...	Cortical ...	27·3	0·365	1·35
Marien Field	Medullary ...	24·1	0·391	1·56
Coarse starch varieties ...	Cortical ...	26·9	0·359	1·34
Berlin Exp. Field ...	Medullary ...	23·1	0·355	1·55
Coarse starch varieties ...	Cortical ...	30·6	0·354	1·17
Marien Field	Medullary ...	27·3	0·383	1·42

East's (6) analyses of two American varieties appeared in 1908.

TABLE IV.

Variety	Zone	% dry matter	% nitrogen in fresh material	% nitrogen in dry material
Rural New Yorker No. 2	Cortical ...	20·95	0·46	2·20
	Outer medullary	18·46	0·47	2·56
	Inner „	14·04	0·45	3·23
Carman No. 3	Cortical ...	22·20	0·49	2·23
	Outer medullary	19·41	0·51	2·63
	Inner „	14·92	0·52	3·49

The results of Waterstradt and Wiflner and of East corroborate those of Coudon and Bussard. The percentage of dry matter decreases from the cortical to the outer and inner medullary layers. Nitrogen does not vary greatly in the fresh material but on the whole the medullary zone is richer than the cortical. This zone therefore contains a considerably higher percentage of nitrogen in its dry matter than does the cortical.

The possible variation in composition between big and little tubers of the same variety must be considered in selecting a sample. Kreusler and Werner(7) maintain that the proportion of dry matter varies little in one variety, and in so far as it does it increases with the size of the tuber. Drechsler and Wollny(8) showed by a great number of experiments that in the same way the proportion of starch varies very little with the size of the tubers. Dry matter determinations by Coudon and Bussard on three sets each consisting of ten whole tubers are as follows:

TABLE V. *Percentage dry matter in big, medium and small tubers.*

Size	Weight Maximum	Minimum	Average	% dry matter
Big	175	91	120	25·97
Medium	86	65	76	26·18
Small	63	28	51	25·03

There is very little variation in the proportion of dry matter in tubers of different size. Coudon and Bussard therefore conclude that in taking a sample, the size of the tubers does not appreciably influence the mean composition of the sample. However they consider it advisable always to use a number of whole tubers in carrying out analyses.

PRESENT INVESTIGATION

The present investigation was carried out at Rothamsted on the variety 'King Edward' grown on the Little Knot Wood field. The soil is clay with flints lying on chalk. It is heavy for potatoes, but the crops are in no way abnormal. During the season under consideration, 1917, the average yield of three varieties was 5 tons per acre, while the average for potatoes in England and Wales was 6·6 tons per acre.

The potatoes were sown during the first week in May and were lifted about the end of September. They were then put in a clamp. The investigation was begun early in 1918. The tubers were taken either directly from the clamp or from a dark cool room to which they were later removed.

METHOD.

The tubers were weighed and their specific gravity determined. They were then divided into zones. The skin was cut off as thinly as possible with a scalpel, but a little of the subcutaneous layer invariably adhered to it. The outer and inner cortical layers each approximately 2·5 mm. thick were removed with a potato peeler. The central area was divided into outer and inner medullary zones.

In some cases the tubers were divided by three parallel cuts at right angles to the main axis into an umbilical, two intermediate, and a terminal region. These were then zoned and put immediately into covered petri dishes for weighing. The drying process was begun in the drying room, average temperature about 30° C., and completed in a steam oven running continuously. The complete process takes from 14–20 days.

Nitrogen was determined by the Kjeldahl method. Preliminary determinations were made on fresh and dried material, but as these are necessarily made on different tubers exact comparison of the results is not possible. The following Table gives the percentage of nitrogen determined in the wet material of the different zones of three separate tubers. It gives for comparison the percentage of nitrogen determined on a sample composed of the dried zones of six tubers, and calculated to the fresh basis.

TABLE VI. *Comparison of Nitrogen determinations made on fresh and dry material of different tubers.*

Zone	% nitrogen determined on wet basis for three tubers			% nitrogen determined on dry basis and calculated to the wet basis
Skin	0·352	0·364	0·373	0·396
Outer cortical	0·356	0·355	0·330	0·349
Inner "	0·326	0·316	0·294	0·325
Outer medullary	0·316	0·355	0·273	0·332
Inner "	0·312	0·369	0·306	0·340

This indicates that there is no great difference in the results obtained by either method. The large bulk of sample necessary when working with fresh material gives much trouble as it tends to froth over. Determinations on fresh material were therefore discontinued.

Samples were prepared by thoroughly mixing together the dried material of corresponding zones of three or six tubers of about the same size. This composite sample was powdered with a pestle and mortar and duplicate samples weighing about 1 gm. taken for nitrogen estimations.

COMPOSITION OF THE TUBER.

Dry Matter.

The average percentage of dry matter in different zones, determined individually on six small, six medium and six large tubers, is:

TABLE VII. *Dry Matter in different Zones of the Tuber.*

	Small 54–84·5 gms.		Medium 139·5–169·2 gms.		Large 184·9–259·9 gms.		Average of 18 tubers
Zone	% of whole	% dry matter	% of whole	% dry matter	% of whole	% dry matter	% dry matter
Skin	2·78	14·29	1·85	15·08	2·83	13·44	14·01
Cortical, outer ...	27·54	24·86	20·29	23·43	18·11	23·36	23·71
,, inner ...	24·68	29·25	20·11	28·72	18·92	27·57	28·30
Medullary, outer ...	31·32	25·76	36·43	25·49	39·95	25·05	25·28
,, inner ...	13·67	20·19	21·32	18·46	20·19	17·48	18·15
[Cortical, outer and inner	52·22	26·93	40·40	26·08	39·03	25·52	26·00]

Detailed results for each tuber are given in Appendix Table I.

The percentage of dry matter in the whole cortical layer has been calculated from the results obtained for inner and outer cortical layers, to facilitate comparison with results obtained by other investigators.

The figures obtained corroborate the results of Coudon and Bussard and other workers. The skin contains the lowest and the cortical layers. taken together, the highest percentage of dry matter. The external medullary zone has a higher percentage dry matter than the internal.

Our figures also show that the inner cortical layer contains a higher percentage of dry matter than the outer cortical. Thus the dry matter increases from the outside of the tuber to the inner cortical layer and then decreases towards the centre. In order to determine where this decrease actually begins a sample of twenty tubers of varying sizes were zoned as before, and in addition a zone adjacent to the inner cortical layer was taken from the outer medullary zone. The corresponding layers from all the tubers were analysed together. The results obtained were:

TABLE VIII.

Zone	% of whole	% dry matter
Skin	4·7	15·50
Cortical, outer ...	19·2	23·33
,, inner ...	19·5	28·40
Medullary, outer, *a*	18·2	27·43
,, ,, *b*	18·7	23·28
,, inner...	19·7	17·78

The decrease in dry matter begins immediately inside the inner cortical layer and proceeds to the inner medullary region.

Comparison of dry matter determinations in different layers of small, medium and large tubers respectively shows in each zone a slight tendency for the smaller tubers to be drier than the larger ones. This is most marked in the inner medullary zone. Appendix Table I shows that while this tendency is perceptible in the average results, it is not found consistently in individual tubers.

A higher percentage dry matter was found in the umbilical region than in the terminal or sprout end. Six tubers divided into the two halves each showed this (Appendix Table II) and gave an average dry matter of 25·16 per cent. in the umbilical and only 22·51 per cent. in the terminal half. Further determinations were made on three large tubers, three medium and three small. Each was divided into four sections by three cuts at right angles to the long axis and each section was zoned (Fig. 1). A sample of 20 tubers was treated in the same way except that a layer 2·5 mm. thick was separated from the outside of the outer medullary zone (Fig. 2).

The percentage of dry matter in individual tubers (Appendix Table III) and in the average (Figs. 1 and 2) is higher in the umbilical than in the terminal half in every zone except the skin, in which there is no consistent variation. In the outer cortical zone dry matter rises from the terminal section 1 to 3 and then either rises or falls to section 4. The increase in proportion of dry matter from the terminal to the umbilical region is most marked in the inner cortical layer where it rises steadily from section 1 to 4. It behaves in the same way in the layer immediately beneath it (Fig. 2). In the medullary zones, especially the inner, the most central region of the zone, that is the part contained in the two intermediate sections, tends to be the wettest. The umbilical medullary region in section 4 is drier than the terminal 1, and section 3 is drier than 2.

Again in each section of every tuber without exception dry matter increases from the skin to the inner cortical layer and then decreases to the inner medullary.

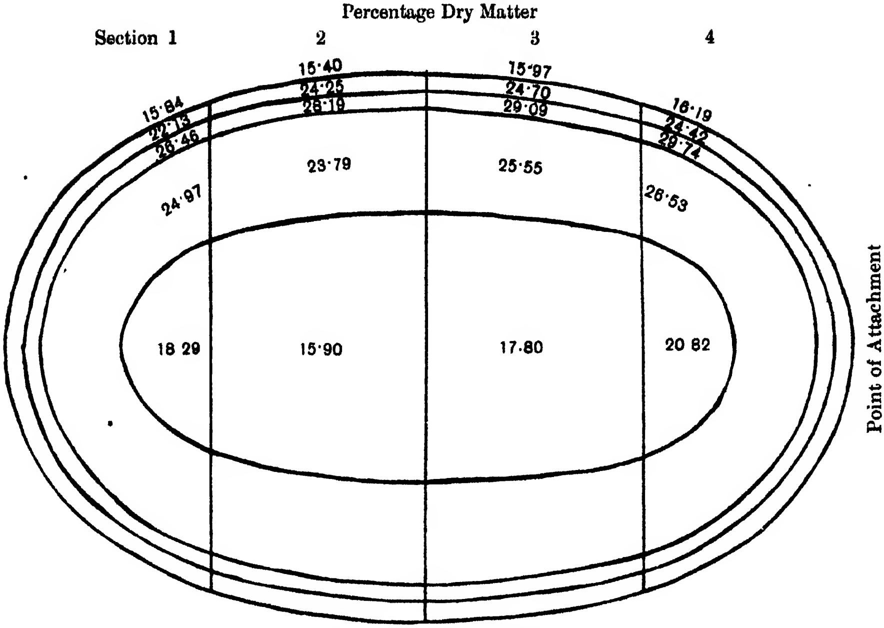


Fig. 1. Average of 9 tubers.

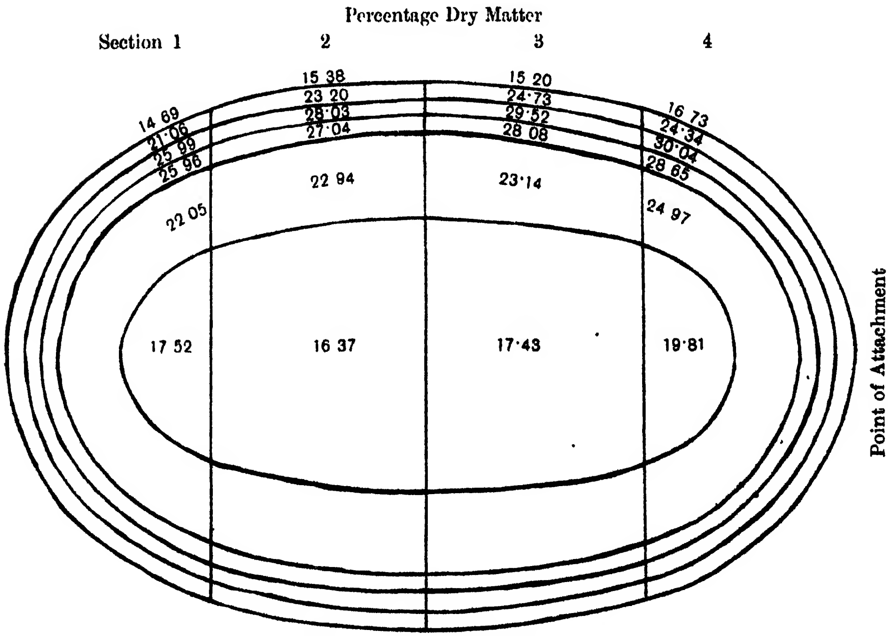


Fig. 2. From sample of 20 tubers.

The Distribution of Nitrogen.

Nitrogen determinations in the different zones of six tubers individually gave the following results:

TABLE IX. *Percentage of Nitrogen.*

Tuber	1		2		3		4		5		6	
Zone	Fresh basis	Dry basis	Fresh basis	Dry basis	Fresh basis	Dry basis	Fresh basis	Dry basis	Fresh basis	Dry basis	Fresh basis	Dry basis
Skin	0·46	3·22	0·42	2·96	0·37	2·62	0·35	2·34	0·36	2·41	0·37	2·48
Cortical, outer ...	0·38	1·53	0·35	1·39	0·39	1·55	0·36	1·52	0·36	1·52	0·33	1·41
,, inner ...	0·33	1·14	0·30	1·04	0·33	1·14	0·33	1·13	0·32	1·10	0·29	1·02
Medullary, outer...	0·37	1·43	0·32	1·24	0·36	1·40	0·32	1·24	0·36	1·39	0·27	1·07
,, inner...	0·40	1·96	0·32	1·57	0·40	1·96	0·31	1·69	0·37	2·00	0·31	1·66

Determinations on two sets of six tubers gave the following averages:

TABLE X.

	Six small tubers		Six medium tubers	
Zone	Fresh basis	Dry basis	Fresh basis	Dry basis
Skin	0·40	2·82	0·40	2·62
Cortical, outer	0·41	1·63	0·35	1·49
,, inner	0·29	1·00	0·33	1·13
Medullary, outer	0·34	1·30	0·33	1·30
,, inner	0·38	1·89	0·34	1·84

The variation of nitrogen in the fresh material of the different zones is relatively small and is not always quite the same in different tubers. On the whole the skin tends to contain the highest proportion and this decreases to the inner cortical layer and increases again in one or both medullary zones. Thus a tendency appears for the percentage of nitrogen in the fresh material to vary from zone to zone in the opposite way to the dry matter, so that the inner cortical, the zone richest in dry matter, is also poorest in nitrogen.

When nitrogen is calculated to the dry basis it follows that the variation from one zone to another is much more markedly in the opposite direction to the dry matter.

The distribution of nitrogen in the zones of the terminal, intermediate and umbilical sections determined on three groups of three tubers whose percentage dry matter is given in Appendix Table III, was

TABLE XI.

	Average of 3 small tubers				Average of 3 medium tubers				Average of 3 large tubers			
	Section				Section				Section			
Zone	1	2	3	4	1	2	3	4	1	2	3	4
Skin	0·40	0·42	1·13	0·42	0·26	0·40	0·45	0·45	0·45	0·36	0·51	0·54
Cortical, outer	0·35	0·36	0·37	0·40	0·33	0·33	0·34	0·37	0·33	0·34	0·35	0·41
inner	0·29	0·29	0·32	0·32	0·29	0·30	0·33	0·35	0·32	0·37	0·35	0·38
Medullary, outer.	0·30	0·32	0·34	0·29	0·30	0·34	0·37	0·38	0·30	0·35	0·44	0·40
inner.	0·33	0·36	0·39	0·40	0·34	0·32	0·36	0·36	0·32	0·32	0·36	0·38

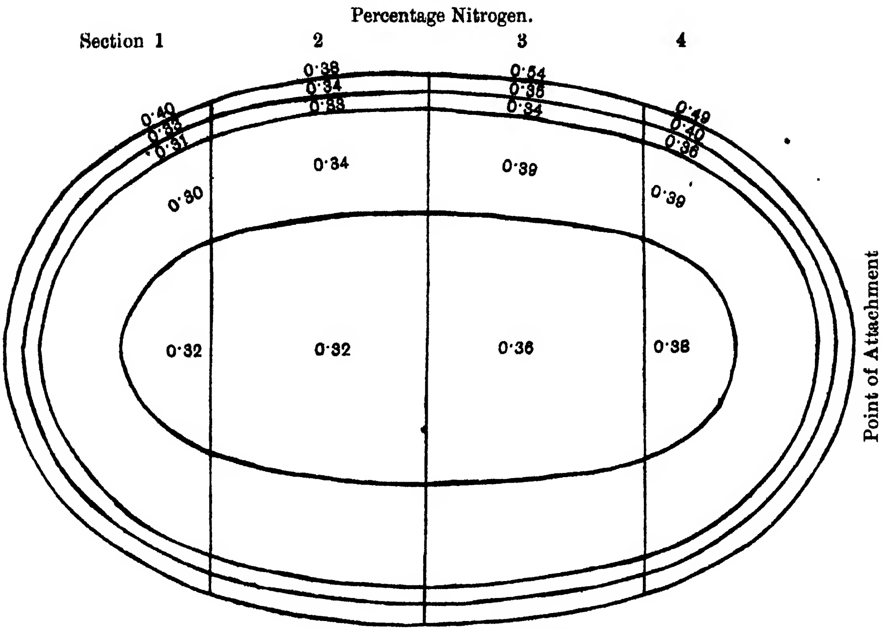


Fig. 3. Average of 9 tubers.

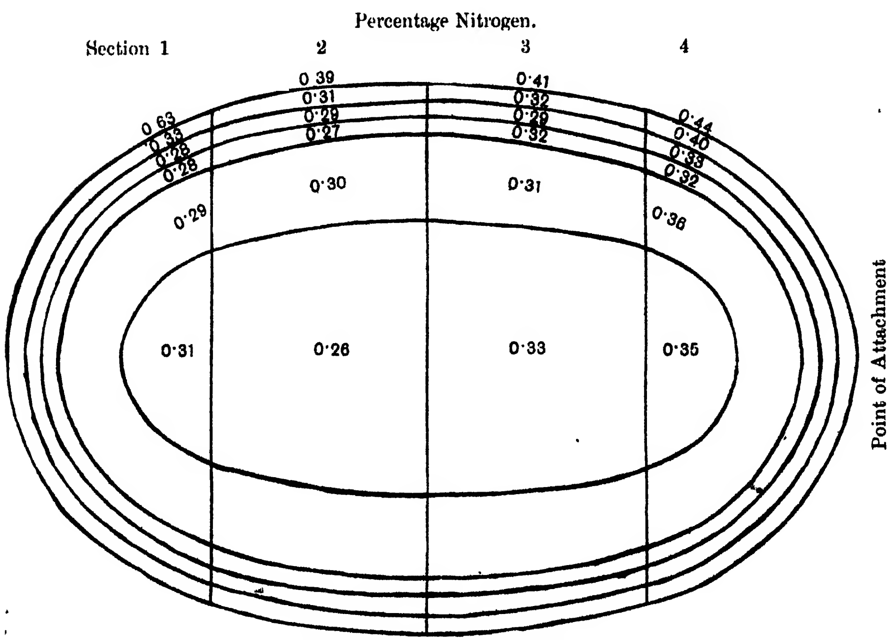


Fig. 4. From sample of 20 tubers.

The average for nine tubers is given in Fig. 3, and Fig. 4 shows the figures obtained for a sample of twenty tubers.

The tendency for the proportion of nitrogen in the fresh material to decrease from the skin to the inner cortical and then to rise is observable in each zone. There is a distinct increase in nitrogen from the sprout end to the point of attachment in each zone. Thus the increase of dry matter from the terminal to the umbilical region is accompanied by an increase in the percentage of nitrogen in the fresh material.

Dry Matter Distribution in relation to the structure of the Tuber.

Figs. 5 and 6 represent respectively transverse and longitudinal sections of the tuber as seen by the naked eye. The vascular bundles stained with phloroglucin show up clearly as a broken ring surrounded

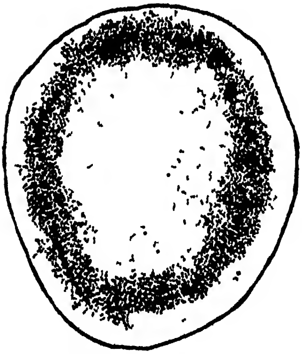


Fig. 5. Transverse section of potato tuber, natural size

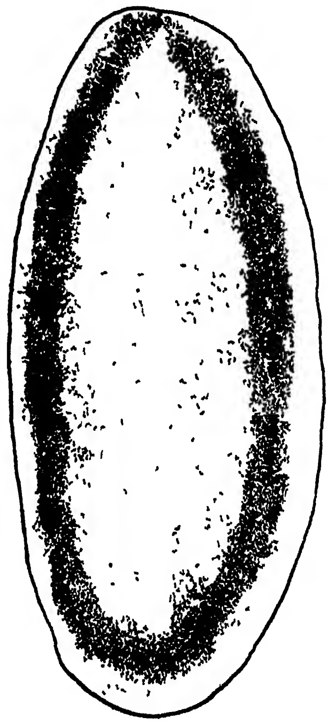


Fig. 6. Longitudinal section of potato tuber, natural size.

by dense-looking tissue. The central translucent part of the tuber with its pith rays penetrating into the outer medullary zone is clearly seen. A section of the tuber magnified eight times is shown in Fig. 7 while

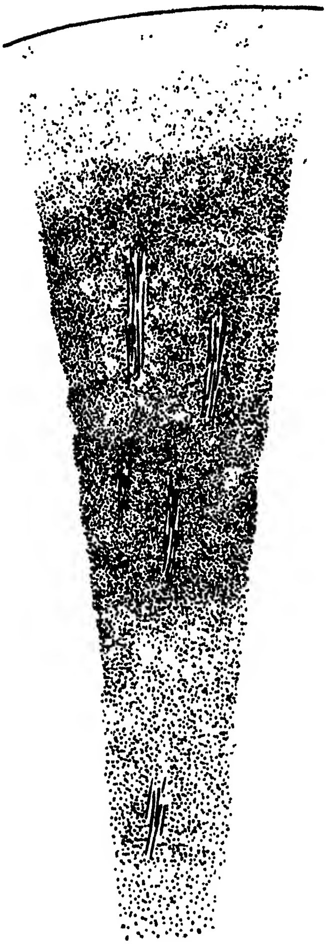


Fig. 7. Transverse section of potato tuber. × 8.

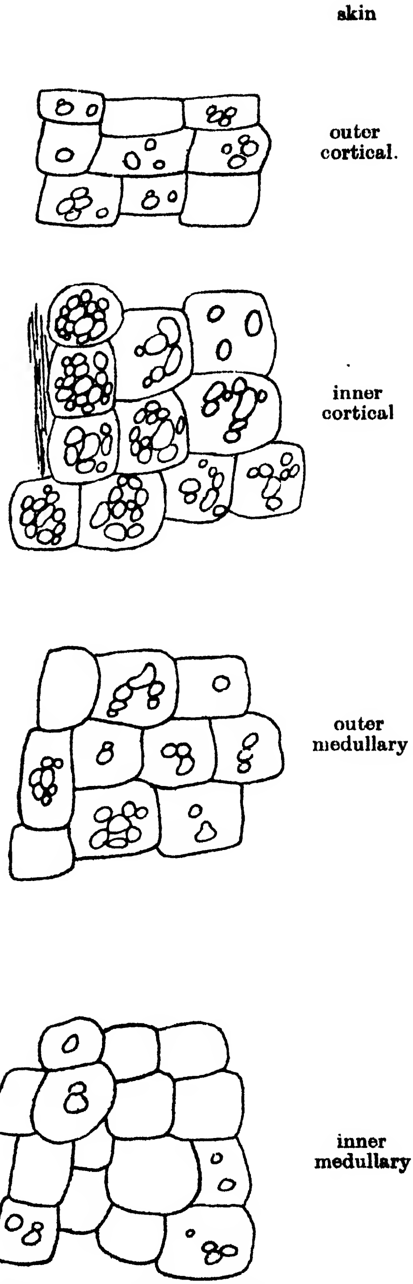


Fig. 8. Groups of cells typical of each zone.

Fig. 8 shows the structure of a few cells typical of each layer. The starch grains are easily seen without staining, but iodine may be used to show them up yet more clearly.

The skin consists of layers of cork cells and probably includes some of the large cells just underneath. These contain a little chlorophil but very little if any starch.

In the outer cortical layer there is a fair amount of starch which increases to a maximum in the inner cortical and then decreases towards the central part of the tuber so that in the inner medullary layer there is very little starch present. The inner cortical layer not only contains the maximum amount of starch, but also the greater part of the vascular system.

The starch grains are densest in the region of the vascular bundles and decrease towards the centre and towards the surface of the tuber. The distribution of dry matter has been shown to be very similar, as it attains its maximum in the inner cortical layer and decreases from this zone towards the skin and towards the inner medullary zone. Thus there appears a very close relation between dry matter and starch content.

In this connection the results which Dr W. E. Brenchley (9) obtained in her study of the wheat grain are of interest. As the grain develops instead of the soluble carbohydrate being carried from the vascular bundle across the width of the endosperm and deposited at the outer edge of the grain, thus leaving a clear passage behind it, the cells nearest the bundle are the first in which starch is deposited. This makes the percolation of reserves more difficult, so that the tendency in the earlier stages is for starch to be densest in the region of the conducting strand and to decrease in the cells further from it in the same way as has been shown in the potato tuber. Later the whole endosperm becomes packed with starch but no comparable stage of maturity is ever reached in the potato tuber.

Dry Matter and Specific Gravity.

The total percentages of dry matter and the specific gravity of 27 individual tubers are given in Table XII.

The specific gravity increases almost invariably with the percentage of dry matter. This consistent relationship is clearly brought out in Fig. 9 in which the specific gravity of each tuber is plotted against the corresponding percentage dry matter. The correlation coefficient is $0{\cdot}972 \pm 0{\cdot}007$.

A further illustration of this close relationship was obtained from

data compiled by Lawes and Gilbert from manurial experiments on potatoes in Little Hoos field during the years 1876-1900. They determined somewhat roughly the specific gravity and the percentage of dry

TABLE XII.

% dry matter	Specific gravity	% dry matter	Specific gravity
21·43	1·0812	24·05	1·0945
21·51	1·0825	24·28	1·0957
21·61	1·0846	24·35	1·0968
22·38	1·0811	24·45	1·0934
22·67	1·0859	24·81	1·0967
22·75	1·0849	25·14	1·0989
23·01	1·0886	25·63	1·1005
23·29	1·0934	25·86	1·1019
23·34	1·0917	26·13	1·1018
23·43	1·0918	26·55	1·1045
23·52	1·0917	26·93	1·1047
23·67	1·0926	27·16	1·1080
23·71	1·0888	27·57	1·1058
23·81	1·0927		

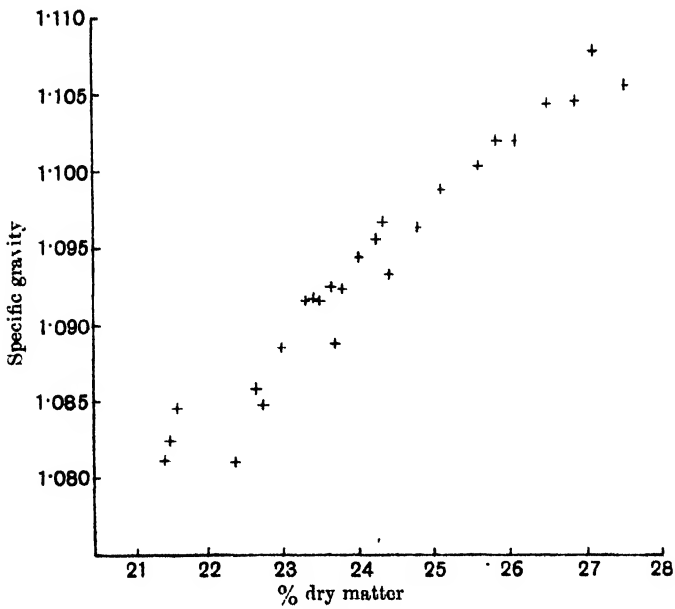
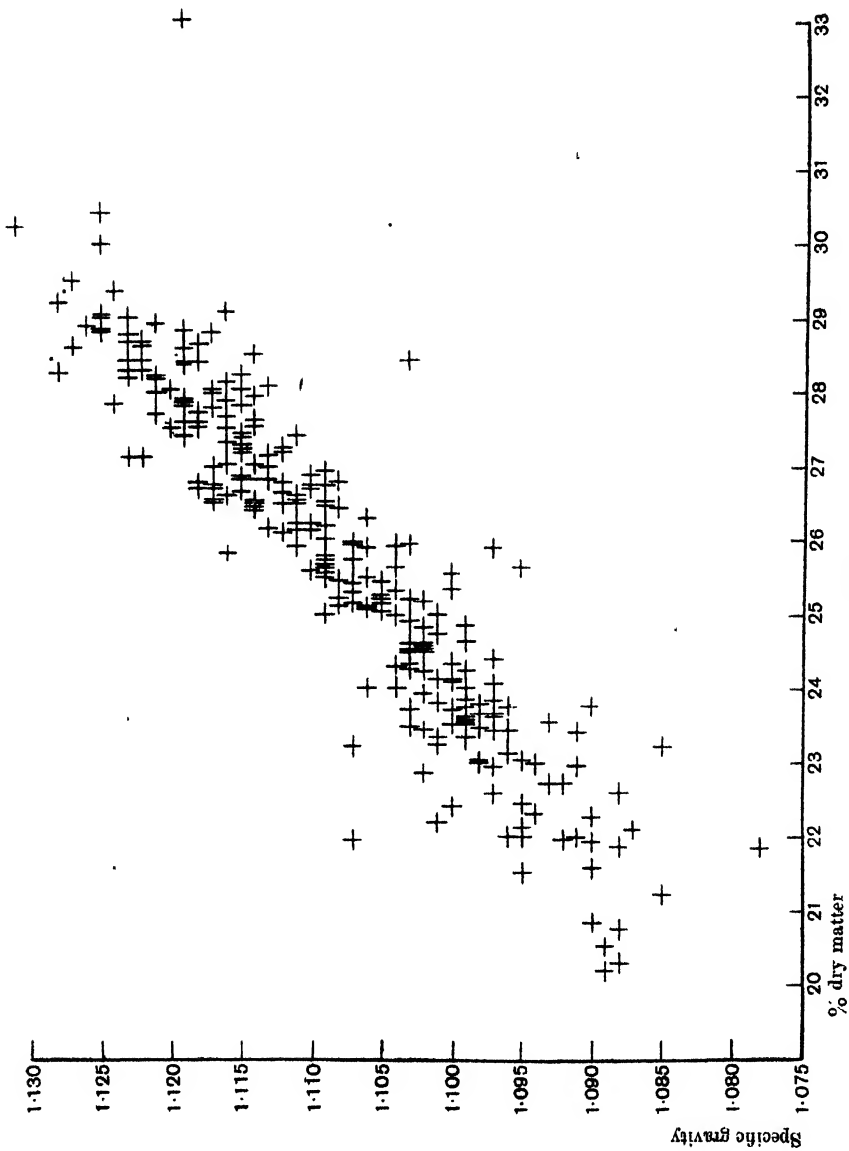


Fig. 9.

matter of 250 samples, each consisting of about 30 tubers. These results are represented graphically in Fig. 10. The correlation coefficient obtained for this series was $+0{\cdot}875 \pm 0{\cdot}010$. In view of the seasonal



Specific gravity
1·130
1·125
1·120
1·115
1·110
1·105
1·100
1·095
1·090
1·085
1·080
1·075
% dry matter
20
21
22
23
24
25
26
27
28
29
30
31
32
33

Fig. 10.

variation and the differences in manurial treatment, this high value suggests that for many purposes determination of the specific gravity may be a sufficiently accurate index of the dry matter content.

For the calculation of the correlation coefficients we are indebted to Mr E. M. Crowther.

Method of Sampling.

The unequal distribution of dry matter and nitrogen makes it difficult to obtain a sample typical of the whole tuber. Coring, whether transverse, longitudinal or diagonal, results in the inclusion of too much of the central wet part of the tuber and so gives too low a figure for the dry matter content. The following table will give some idea of the error involved. In six tubers treated individually dry matter was estimated in one quarter and in a longitudinal core taken through half the tuber (Fig. 11).

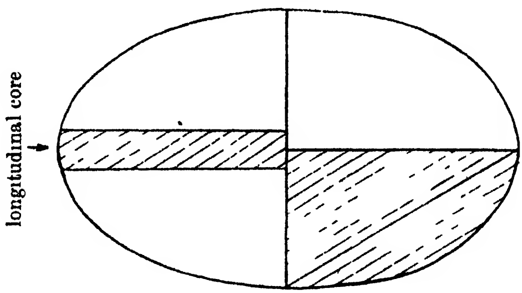


Fig. 11.

TABLE XIII.

Part of tuber	Percentage of dry matter						Average of 6 tubers
	Tuber 1	2	3	4	5	6	
One quarter	24·07	19·33	21·39	18·43	21·34	24·04	21·43
Longitudinal core	15·59	18·05	15·67	14·63	12·13	18·79	15·81

In six other tubers a diagonal and a transverse core were taken instead of a longitudinal one (Fig. 12).

TABLE XIV.

Part of tuber	Average dry matter						Average of 6 tubers
	Tuber 1	2	3	4	5	6	
One quarter	22·88	23·80	22·88	21·19	22·67	23·32	22·79
Diagonal core	18·90	22·16	18·91	18·08	17·34	19·29	19·11
Transverse core	21·65	18·38	17·45	18·47	18·77	19·31	19·00

In each tuber the core whether longitudinal, diagonal or transverse contains a distinctly smaller proportion of dry matter than the quarter which is a more nearly representative sample of the whole tuber.

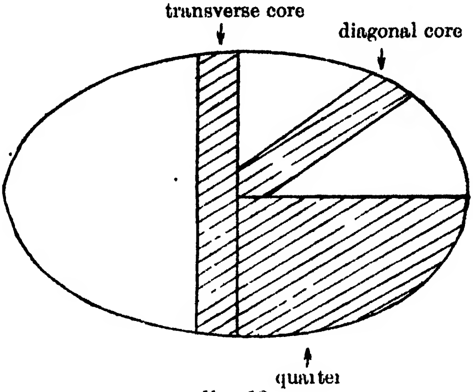


Fig. 12.

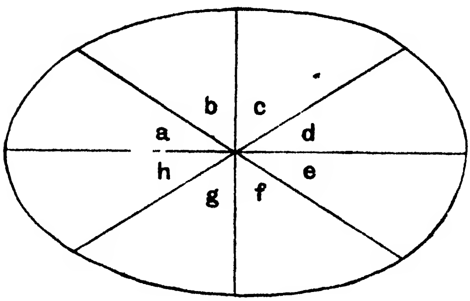


Fig. 13.

Division of the tuber into sectors as in Fig. 13 gave the following results:

TABLE XV.

Sector	% dry matter	Sectors	% dry matter	Sectors	% dry matter	% dry matter of whole tuber
a	22·82	*a*+*e*	22·89	*a*+*c*+*e*+*g*	22·84	22·66
b	21·77	*b*+*f*	21·86	*b*+*d*+*f*+*h*	22·47	
c	23·14	*c*+*g*	22·79			
d	24·43	*d*+*h*	23·09			
e	22·94					
f	22·00					
g	22·37					
h	21·99					

When one sector is taken as a sample there may be a difference from the mean of nearly 2 per cent. dry matter (sector *d*). When radially

opposite sectors are taken this is reduced to less than 1 per cent., while if four alternating sectors are taken the error becomes negligible. The obvious causes of error in the sector method are the difficulty of cutting a sector the apex of which is exactly in the middle of the tuber, and the fact that the composition of the tuber is different at either end. Both these errors may be counterbalanced by taking radially opposite sectors.

Another method is to cut the tuber into eighths by two cuts at right angles to each other along the long axis and one cut along the transverse axis of the tuber. Two diagonally opposed eighths taken as a sample gave the following result in three tubers.

TABLE XVI. *Percentage Dry Matter in diagonally opposed $\frac{1}{8}$ths.*

Pairs of eighths	1	2	3	4	Average for whole tuber
Tuber 1	26·54	26·94	27·10	26·73	26·77
„ 2	23·40	22·84	23·71	23·40	23·23
„ 3	21·97	22·18	21·83	21·48	21·91

The greatest difference from the average percentage dry matter is 0·5 per cent.

SUMMARY.

1. The percentage of dry matter in the potato tuber is lowest in the skin and increases to the inner cortical layer, the zone containing the greater part of the vascular system, and decreases towards the centre of the tuber.

2. In each zone the proportion of dry matter is higher towards the umbilical than the terminal end of the tuber.

3. The percentage of nitrogen in the fresh material tends to decrease from the skin to the inner cortical layer and to increase in the medullary zone. Thus it increases from zone to zone in the opposite direction to the dry matter.

4. Nitrogen tends to increase with dry matter from the terminal to the umbilical end.

5. Microscopical examination shows the starch grains densest in the region of the vascular system, and decreasing towards the centre and surface of the tuber.

6. A high degree of correlation is found between the specific gravity and percentage dry matter of whole tubers.

7. For purposes of sampling the method of taking two radially opposed sectors, or two diagonally opposed eighths, was far more accurate than the coring method.

APPENDIX.

Table I. *Distribution of Dry Matter in different Zones of Tuber.*

Weight tuber gms.	Skin	Outer cortical	Inner cortical	Outer medullary	Inner medullary	Total % dry matter in tuber
	Percentage dry matter					
Small Tubers.						
54·0	15·71	26·75	31·70	27·81	22·20	27·57
64·4	13·03	21·57	24·40	22·21	16·01	21·51
71·4	13·06	24·53	28·28	24·42	18·89	24·45
74·0	13·46	26·01	30·03	26·67	21·70	26·13
78·7	15·70	24·47	31·02	27·86	21·67	26·55
84·5	16·05	25·64	30·02	25·59	20·32	25·63
80·3*	16·30	19·48	25·24	23·18	17·25	21·43
82·7*	13·94	26·52	31·71	27·84	21·56	27·16
89·7*	16·66	25·77	30·68	28·82	22·76	26·93
Medium Tubers.						
139·5	13·97	23·45	27·97	24·89	19·13	23·81
140·8	15·59	21·24	29·03	24·88	17·24	23·29
152·1	14·23	22·10	26·87	24·17	16·92	22·67
157·2	16·82	24·31	29·31	26·12	19·49	24·81
164·0	15·86	24·70	30·24	27·35	19·80	25·14
169·2	14·74	24·51	28·97	25·42	17·44	24·28
134·7*	14·29	23·95	27·71	23·22	15·25	23·34
135·7*	18·80	25·90	31·01	25·76	18·41	25·86
140·4*	14·78	23·82	28·56	24·20	16·90	24·15
Large Tubers.						
184·9	14·30	23·56	27·20	24·88	17·86	23·43
199·9	13·17	23·05	28·23	26·12	17·18	24·05
226·6	13·92	23·91	28·26	25·34	18·37	23·71
227·7	13·19	23·19	27·43	24·60	16·92	23·01
229·5	11·69	21·73	26·17	23·35	15·70	21·61
259·9	14·07	24·60	28·17	26·00	18·71	24·35
297·8*	15·84	22·53	26·80	23·63	15·97	22·38
351·9*	15·04	24·21	28·16	25·63	17·56	23·67
391·9*	16·81	23·83	27·91	25·16	17·35	23·52

* Results calculated from figures for App. Table III.

Table II. *Percentage of Dry Matter in the terminal and umbilical halves of six large tubers.*

	Terminal half	Umbilical half	Whole tuber
	20·47	22·68	21·54
	21·93	24·42	23·17
	22·43	24·19	23·29
	22·31	25·33	23·57
	23·51	27·47	25·31
	24·45	26·89	25·63
Average	22·51	25·16	23·75

TABLE III. *Distribution of Dry Matter in the Potato Tuber.*

	Small tubers				Medium tubers				Large tubers			
Weight, tuber ...	89·73 gms.				140·4 gms.				391·9 gms.			
Specific gravity ...	1·105				—				1·091			
Total % dry matter	26·93				24·15				23·52			
Section	1	2	3	4	1	2	3	4	1	2	3	4
Skin	18·31	12·65	19·48	16·77	15·00	14·75	15·05	14·29	15·55	16·12	17·98	18·13
Cortical, outer ...	22·93	25·07	26·34	28·00	22·90	24·37	24·38	23·51	21·91	24·42	24·43	25·10
,, inner ...	27·48	29·60	31·27	33·36	27·61	28·91	28·92	28·67	25·90	27·46	28·88	30·69
Medullary, outer ...	27·39	28·02	29·05	31·20	23·28	22·40	25·32	26·54	25·28	23·02	26·58	27·30
,, inner ...	23·71	20·95	22·28	27·01	16·60	15·74	17·60	19·61	17·56	15·64	18·53	20·18
Weight, tuber ..	82·60 gms.				135·7 gms.				351·9 gms.			
Specific gravity ...	1·109				1·103				1·092			
Total % dry matter	27·16				25·86				23·67			
Section	1	2	3	4	1	2	3	4	1	2	3	4
Skin	15·05	13·46	13·34	13·95	17·68	20·00	19·75	18·52	16·08	14·67	14·72	14·77
Cortical, outer ...	24·36	26·94	27·35	26·97	23·80	25·74	26·95	27·10	21·83	24·53	25·00	25·36
,, inner ...	29·03	30·83	33·12	33·51	29·12	30·68	32·02	32·09	25·86	28·00	28·63	30·41
Medullary, outer ...	26·23	26·19	29·16	30·85	25·64	24·64	25·71	27·67	26·20	24·32	26·06	27·13
,, inner ...	21·45	19·56	21·67	26·36	19·04	17·08	18·47	22·36	20·18	15·51	17·06	21·47
Weight, tuber ...	80·25 gms.				134·7 gms.				297·8 gms.			
Specific gravity ...	1·081				1·091				1·081			
Total % dry matter	21·43				23·34				22·38			
Section	1	2	3	4	1	2	3	4	1	2	3	4
Skin	17·34	20·34	12·73	14·94	14·38	13·63	14·87	14·29	15·61	15·53	15·48	16·81
Cortical, outer ...	18·27	20·33	19·06	20·16	23·58	24·79	24·98	22·02	21·16	22·52	23·40	22·89
,, inner ...	23·33	25·74	26·29	25·96	28·19	29·11	27·81	25·42	25·39	26·51	27·12	28·35
Medullary, outer ...	23·06	23·30	23·10	23·18	24·49	23·64	22·44	22·17	23·25	22·39	23·95	25·49
,, inner ...	18·40	16·42	16·89	18·78	17·51	14·50	14·87	16·24	16·16	14·13	16·29	18·78
	Average for 3 small tubers				Average for 3 medium tubers				Average for 3 large tubers			
Section	1	2	3	4	1	2	3	4	1	2	3	4
Skin	16·84	14·87	16·22	15·60	15·70	15·63	16·20	15·52	15·73	15·45	16·12	16·69
Cortical, outer ...	21·65	24·47	25·03	24·92	23·41	24·94	25·37	24·02	21·69	23·89	24·24	24·44
,, inner ...	26·45	29·09	30·94	31·01	28·25	29·53	29·57	28·70	25·76	27·36	28·26	29·84
Medullary, outer ...	25·26	26·04	27·24	27·70	24·47	23·59	24·51	25·64	25·07	23·35	25·55	26·63
,, inner ...	21·21	19·14	20·33	24·05	17·94	15·81	17·04	19·48	17·95	15·24	17·35	20·24

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(*Received March 28th*, 1919.)

FURTHER STUDIES ON THE SOILS OF NORTH WALES.

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In a former paper, one of us has discussed the Palaeozoic soils of North Wales[1]. In that paper, the Carboniferous soils were omitted as being rather apart from the other Palæozoic soils, which formed a convenient group for study by themselves. In the present paper, the authors[2] discuss Carboniferous soils and the soils derived from the Northern Drift, together with the associated deposits of more recent origin.

Referring to the orographical map, it may be stated that the soils examined in the present paper occur west of *AB* and north of *CDEFGH*. They are thus complementary to the soils described in the previous paper. The region in question comprises west Carnarvonshire, portions of eastern Anglesey, the coastal districts of east Carnarvonshire and Denbighshire, the Vale of Clwyd, most of Flintshire and the eastern fringe of Denbighshire.

The physical features and climate have already been described and are sufficiently obvious from the accompanying orographical and rainfall[3] maps. The soils of the present paper are generally lowland soils under rainfalls which, for North Wales, must be reckoned as fairly low. Although the district is mainly lowland, it is, as a rule, undulating, with the exception of the broad Vale of Clwyd, the east of Flintshire and the coastal fringe. The climate of west Carnarvonshire is considerably wetter than that of the eastern regions. The winters are also milder and the summers cooler, so that while the climatic conditions of the

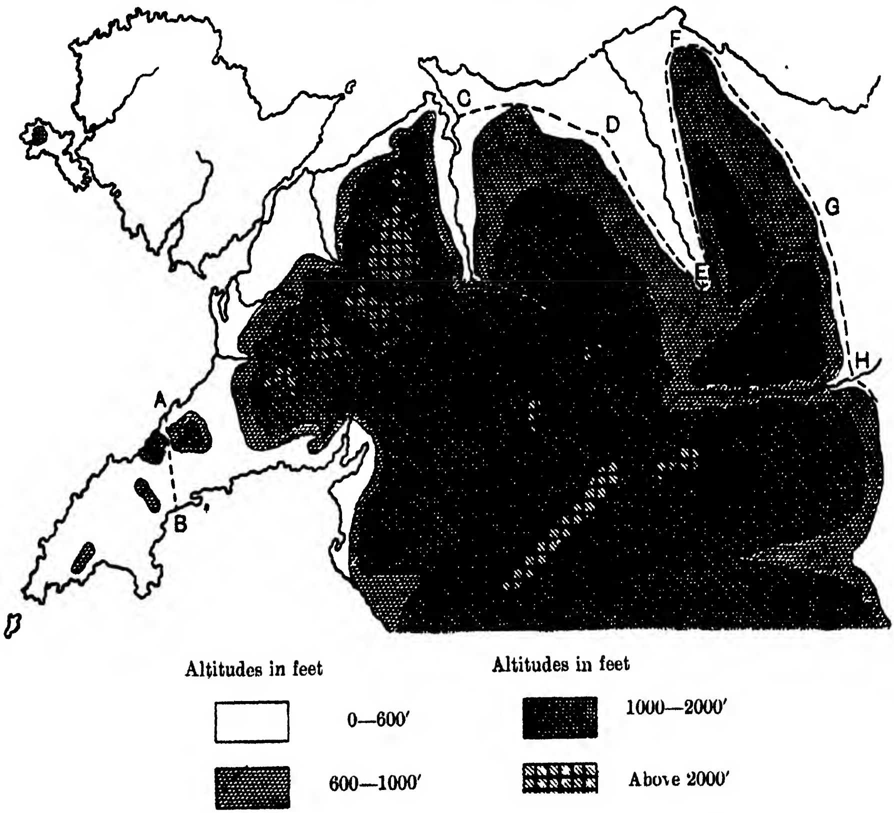
[1] "Studies on the Palaeozoic Soils of North Wales," by Gilbert Wooding Robinson. This *Journal*, **8**, Part III, June, 1917.

[2] Flintshire, C. F. Hill; Anglesey, Carnarvonshire and Denbighshire, G. W. Robinson.

[3] The rainfall map given in the previous paper contained certain inaccuracies which have now been rectified.

eastern regions approach to those of the adjoining English counties, the western region is more typically North Welsh. These factors are of considerable influence on the soils of the respective regions.

North Wales. Orographical.

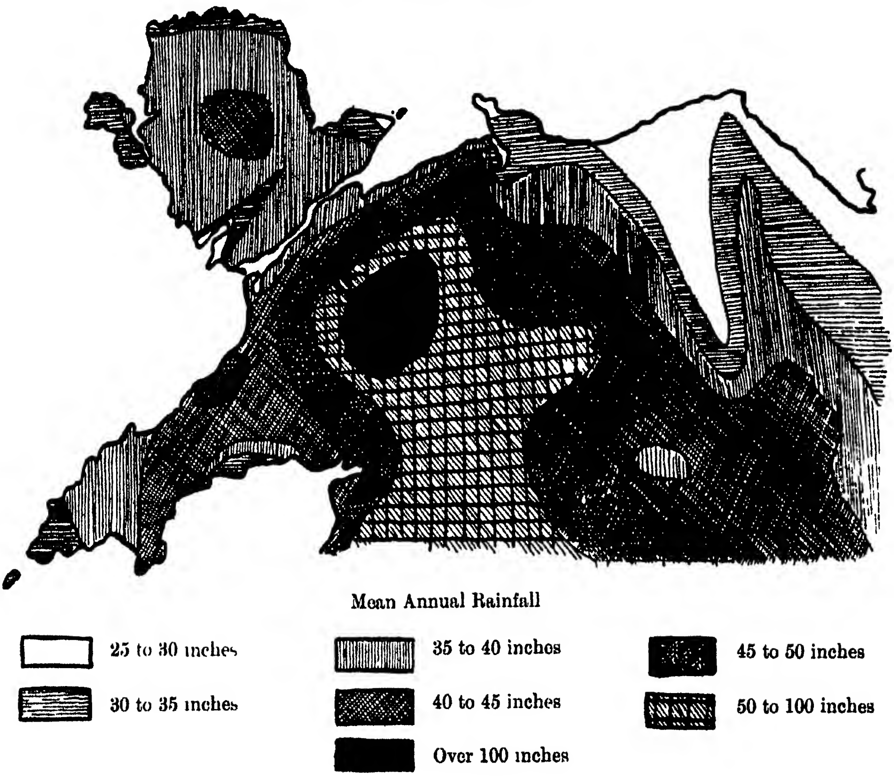


Geology.

The region dealt with in the present paper is mainly drift covered. The only sedentary soils described are those lying on the Carboniferous Limestone and Millstone Grit. The former rocks occur in the eastern parts of Anglesey, the Creuddyn peninsula of Carnarvonshire, along the west of the Vale of Clwyd and on a broad outcrop north and east of the Clwydian hills stretching from the coast nearly to the Dee valley. In many places it is covered with drift, so that the development of Carboniferous soils is by no means so extensive as would appear from

the geological map. Millstone Grit and Gwespyr Sandstone are found to a small extent in Flintshire to the east of the Carboniferous Limestone. Coal Measures are found over a wide area of Flintshire and Denbighshire, but are generally obscured by drift. The Permian and Triassic formations are also generally drift covered. Isolated outcrops of Trias occur near Ruthin and Denbigh[1].

NORTH WALES. Rainfall.



The chief feature of our region is, however, its glacial geology. North Wales has been subjected to two glaciations, namely the Northern glaciation running roughly N.E. to S.W. and the Welsh glaciation radiating from the mountain areas. The deposits from the two glaciations are quite distinct. While the Welsh glacial drift consists mainly of local material scraped down from the uplands, the Northern drift

[1] We have not examined any sedentary soils from the Trias. For a description of Triassic soils, see *A Survey of the Soils and Agriculture of Shropshire*, by G. W. Robinson. Shrewsbury, 1913.

TABLE I. *Average percentage composition of soil types.*

	Carboniferous Limestone Soils				West Carnarvonshire Light Loam		Sands and Gravels			
	Anglesey and Carnarvon		*Denbigh and Flint*				*West Carnarvonshire*		*Eastern*	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
Soils:										
Fine Gravel ..	4·0	·94– 9·48	5·7	3·53– 9·32	6·4	1·26–11·65	6·9	2·83–14·02	3·7	1·09– 8·04
Coarse Sand ..	20·3	7·58–29·15	20·0	8·88–29·03	16·3	8·83–24·84	38·4	19·85–65·86	45·9	29·60–63·38
Fine Sand	26·3	16·56–35·94	17·8	9·80–24·55	25·7	19·70–35·40	22·1	9·67–30·64	18·7	12·46–23·84
Silt	12·8	9·84–21·45	14·9	11·47–20·07	15·0	10·52–20·00	10·8	5·08–17·73	8·3	5·80–12·73
Fine Silt	16·2	12·97–21·72	21·3	12·80–33·16	17·4	14·23–21·32	9·2	2·93–16·73	10·7	5·69–16·23
Clay	5·6	3·32– 8·64	7·3	3·89–11·59	4·2	2·10– 6·97	2·3	·78– 3·04	3·5	1·81– 6·78
Moisture	2·7	1·80– 5·14	2·5	·68– 4·70	3·0	1·30– 7·00	2·2	1·08– 4·30	1·5	·66– 2·58
Organic Matter ..	8·3	6·08–10·12	8·8	5·58– 8·46	9·3	6·66–13·16	7·3	5·50–10·70	6·6	3·28–10·26
Nitrogen	·27	·209–·340	·22	·180–·288	·315	·204–·418	·26	·142–·325	·235	·125–·568
Potash (K_2O) sol. in HCl	·47	·276–·640	·590	·476–·612	·445	·306–·595	·36	·198–·612	·395	·320–·568
Phosphoric Acid (P_2O_5) sol. in HCl	·13	·057–·302	·113	·068–·154	·152	·083–·207	·135	·055–·202	·125	·071–·197
Subsoils:										
Fine Gravel ..	4·5	1·90–11·69	8·6	3·77–13·55	6·8	1·35–11·38	6·7	1·65–19·87	4·2	·36– 7 44
Coarse Sand ...	20·2	8·42–28·42	22·2	13·02–31·98	15·1	8·18–23·96	36·8	19·37–68·98	49·1	31·27–60·60
Fine Sand	26·0	12·74–41·02	17·5	9·83–20·87	27·1	20·68–42·06	24·9	9·75–41·34	18·8	12·76–24·10
Silt	13·2	9·37–18·60	14·4	9·80–19·91	18·3	13·89–25 90	14·0	6·71–22·53	8·9	4·76–12·42
Fine Silt	17·3	13·30–19·26	19·0	15 83–29·12	14·1	6·90–22·54	7·6	1·23–15·17	9·3	4·93–15·76
Clay	8·1	2·41–13·57	8·5	4·43–14·26	5·2	3·01– 9·42	2 9	·76– 6·44	3·9	1·40– 8·37
Moisture	2·1	·96– 5·12	1·5	·50– 3·48	2·3	1·08– 5·14	1 8	·82– 4·22	1·0	·54– 2·06
Organic Matter ..	5·8	4·80– 8·38	5·3	4·24– 5·81	6·3	4·08–11 48	4·9	2·70– 7·26	4·2	1·90– 7·34

HEAVIER DRIFT SOILS

	Transition		Eastern Main Type		Maelor		Vale of Clwyd		Millstone Grit	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
Soils:										
Fine Gravel ...	7·4	4·89–10·41	2·9	1·09– 5·64	2·2	1·81– 2 69	2·0	·92– 2·86	5·5	3·09– 9·27
Coarse Sand ...	26·8	25·24–27·90	24·9	16·71–32·08	10·4	7·93–13·17	24·2	12·31–33·61	20·9	14·35–32·24
Fine Sand	19·0	16·81–21·67	20·3	18·81–24·84	11·7	10·12–12·92	17·8	10·84–21·43	22·5	18·54–25·66
Silt	12·8	11·22–14·44	12·9	10·42–16·36	13·3	13·05–13·72	12·4	10·91–16·96	14·5	12·41–16·72
Fine Silt	14·7	11·87–16·23	17·2	12·21–26·30	30·6	27·45–35·25	19·1	13·25–34·44	16·1	8·99–21·78
Clay	4·7	2·80– 6·79	8·9	7·32–14·44	14·8	12·02–16·47	10·6	7·85–14·89	5·0	3·21– 6·42
Moisture	1·9	1·58– 2·20	2·3	1·38– 4·38	4·3	3·04– 5·40	2·4	1·20– 3·98	3·7	·90– 7·40
Organic Matter ...	8·9	7·26–10·44	7·9	6·02–10·82	10·4	9 86–11·36	7·6	5·28–13·24	9·1	5·78–15·38
Nitrogen	·275	·238–·309	·235	·180–·366	·33	·322– ·330	·22	·117–·460	·285	·196–·393
Potash (K_2O) sol. in HCl	·48	·459–·500	·52	·435–·601	·95	·819–1·050	·65	·534–·860	·45	·343–·554
Phosphoric Acid (P_2O_5) sol. in HCl	·17	·135–·215	·12	·092–·144	·11	·107– ·184	·095	·067–·137	·13	·092–·195
Subsoils:										
Fine Gravel ...	9·4	7·14–12·75	3·7	·75– 9·43	2·5	1·60– 3·33	2·3	1·34– 3·90	9·1	5·11–13·95
Coarse Sand ...	25·4	24·81–26·52	24·7	12·65–32·40	9·9	8·03–11·74	20·7	8·98–25·75	21·1	13·61–31·55
Fine Sand	20·4	20·28–21·40	19·5	15·18–22·49	10·3	6·76–14·84	16·3	8·90–20·30	21·9	18·94–25·40
Silt	14·0	11·30–17·98	13·2	11·38–18·89	13·2	12·22–14·26	11·8	10·32–15·75	18·4	16·06–21·38
Fine Silt	13·8	10·44–16·05	16·7	10·77–24·55	29·6	25·55–35·15	19·7	14·92–29·88	12·7	3·69–20·16
Clay	5·8	3·52– 7·04	15·0	9·85–21·08	24·3	22·47–27·06	18·1	12·34–27·02	7·4	3·40–13·67
Moisture	1·3	1·10– 1·64	1·7	1·00– 2·46	1·7	1·40– 2·84	2·2	1·16– 4·86	1·5	·58– 2·24
Organic Matter ...	6·2	5·36– 7·32	6·6	4·02– 5·62	5·9	5·54– 6·26	4·9	3·54– 5·90	5·1	3·96– 5·56

consists of material generally external to the district but in all cases originating from rocks lying to north or north-east. The drift soils described in the present paper are derived from this external drift. It may be added that in Anglesey there are drift deposits formed by the northern glacier which consist almost entirely of local schistose material, probably transported as ground moraine from no great distance. The soils derived in this way were grouped and studied with the Palæozoic soils. The drift soils of this paper consist mainly of material derived from localities other than those in which they occur. In west Carnarvonshire, however, there is an admixture of local material which makes classification rather difficult. In fact the drifts of that area show all gradations between soils consisting wholly of external material and soils consisting of local material. The typical Northern Drift soil can be readily recognized by the nature of its sand fractions, which consist of rounded quartz grains, while the local material is represented by angular or lenticular fragments of igneous or shaly rocks.

The boundary between northern and local drift is not always well defined. In fact, large areas have been subjected to both glaciations and northern and local drift may be found in vertical succession. Denudation by weathering has led to the surface soil being a mixture of both kinds of drift.

The coastal drift plain of the northern coast of Carnarvonshire and Denbighshire extended much farther north in former times. The vast sand stretches at the northern end of the Menai straits are most probably the remains of former land, for there are no sand bearing rivers which could account for such extensive deposits. This hypothesis, it may be added, is in accord with Welsh traditions.

General.

The results of the analyses are summarised in Table I. In the former paper, it was possible to give some idea of the dispersion of the results in two of the types examined. The soils studied in the present paper do not lend themselves to such treatment. Each type is to be regarded rather as a series varying between limits: one type frequently shades off into another, so that it is not always easy to determine to what type or class a soil should be referred. For example, in the case of the soil type described as the West Carnarvonshire Light Loam, examples are found which genetically and intrinsically approach the Carnarvonshire Stony Loam described in the former paper. The fact that the area examined in this paper is almost entirely covered with drift ex-

plains why the drawing of boundaries is so difficult. There is not a very striking difference between the most common soil types and it would be necessary to make a field to field survey to define them with any degree of precision. The task for the present is to isolate and study the types. Their accurate delineation must be a later and longer task. The future work on these soils will consist, firstly, in a study of the principal types and their peculiarities and, secondly, in mapping out their boundaries on a more detailed scale.

1. *Carboniferous Limestone Soils.*

These soils present some difficulty in classification. There are certain drift soils which do not lie on the limestone at all but which are in all respects similar to true limestone soils and are, in fact, composed of the decomposition products of Carboniferous Limestone. It has been thought well for the present to include these with true limestone soils.

The type occurs in Anglesey, northern Carnarvonshire, both sides of the Vale of Clwyd and over a considerable tract of Flintshire. In the latter area the formation is much obscured by drift. Drift soils of limestone character are found in the eastern corner of Anglesey.

The type is not sufficiently developed to have a characteristic agriculture. It may be noticed, however, that the districts where these soils are cultivated are marked by better grass land than on the other soil types. In Anglesey, it is remarkable that on the limestone, particularly in the northern portion, small holdings and small fields are the rule. It would seem that these areas were enclosed at an early period. While the cultivated soils on the limestone are of considerable fertility, it must also be remarked that there are large tracts only used for rough grazing, such as the Great Orme near Llandudno and Halkyn Mountain in Flintshire. This must be attributed to the elevation and also to the well-known dryness of the formation. Where cultivated, these soils seem to be generally sandy loams of a reddish or light brown colour.

It appears that the soils of Anglesey and Carnarvon are rather more sandy than those of Flint and Denbigh. We have accordingly separated them for the purpose of averaging. It will be seen that the difference is not very striking, but the soils of the former area show more fine sand and less of the finer fractions. They are also rather poorer in potash and richer in phosphoric acid. It must be owned that the Carboniferous soils form a very variable type and the regional classification here adopted does not help matters very much. Probably when a larger number of examples have been examined it may be

Carboniferous Limestone Soils

Anglesey and Carnarvon

		Penmon 4		*Penhwnllys* 10		*Llgwy* 31		*Tynygongl* 12		*Tan-y-Graig* 27		*Llangoed* 38		*Bryn Sieneyn* 39		*Llandudno* C 20		*Gt Orme* C 52		*Gt Orme* C 53	
		Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil
	Fine Gravel ...	1·24	4·26	3·20	4·12	1·56	2·22	1·69	1·46	5·62	4·20	4·97	7·91	9·48	11·69	6·50	5·16	4·61	3·35	·94	1·90
	Coarse Sand ...	25·52	28·24	27·17	24·98	26·27	26·97	20·19	20·36	19·44	15·20	29·15	28·42	15·50	16·39	22·59	23·65	7·58	8·42	9·27	9·19
	Fine Sand ...	22·24	22·00	23·61	23·35	34·45	35·51	29·49	32·57	16·56	12·74	25·56	23·98	26·33	25·31	19·14	10·75	29·71	32·97	35·94	41·02
	Silt	10·05	10·38	10·38	9·37	11·50	11·22	10·80	11·35	13·33	12·63	9·84	10·15	13·08	15·79	10·56	14·67	17·49	18·04	21·45	18·60
	Fine Silt ...	16·53	16·22	14·92	18·82	12·97	13·30	18·88	19·26	21·72	25·84	14·81	15·00	17·96	17·43	14·47	14·30	18·00	18·53	13·60	14·90
	Clay	3·39	8·01	6·38	9·92	3·32	2·41	4·76	5·66	4·44	13·57	6·09	9·55	5·44	5·46	7·42	10·88	8·64	8·94	5·78	6·42
	Moisture ...	5·14	3·12	2·60	2·42	1·96	1·26	2·98	1·48	4·90	5·12	1·80	1·42	1·72	1·20	2·00	2·26	1·86	1·38	2·06	·96
	Organic Matter	8·48	5·14	6·30	4·80	7·30	1·92	7·76	5·66	9·92	7·28	6·08	4·84	9·44	6·00	10·12	8·38	8·50	5·52	9·26	5·46
	Calcium Carbonate ...	Nil	Nil	·52	·27	Nil	Nil	1·26	·76	1·06	·99	Nil	Nil	·74	·34	4·80	5·40	Nil	Nil	Nil	Nil
	Nitrogen ...	·291	—	·209	—	·247	—	·240	—	·333	—	·209	—	·276	—	·340	—	·284	—	—	—
48 hours digestion with HCl	Potash (K_2O) ...	·640	—	·564	—	·310	—	·363	—	·339	—	·610	—	·420	—	·672	—	·342	—	·445	—
	Phosphoric Acid (P_2O_5) ...	·057	—	·123	—	·125	—	·156	—	·132	—	·095	—	·165	—	·302	—	·105	—	·063	—
	Calcium Oxide (CaO) ...	·29	—	·50	—	·24	—	1·31	—	1·35	—	·28	—	·90	—	—	—	·54	—	·18	—
	Magnesium Oxide (MgO)	·33	—	·13	—	·31	—	·29	—	1·31	—	·40	—	·70	—	—	—	·43	—	·50	—
	Insoluble ...	79·8	—	80·0	—	84·2	—	78·1	—	71·0	—	81·6	—	73·9	—	71·2	—	78·8	—	79·9	—
Soluble in 1% Citric	Potash (K_2O) ...	·020	—	·014	—	·027	—	·018	—	·010	—	·014	—	·012	—	·019	—	·018	—	·015	—
	Phosphoric Acid (P_2O_5) ...	·008	—	·021	—	·007	—	·023	—	·012	—	·009	—	·008	—	·078	—	·010	—	·006	—

CARBONIFEROUS LIMESTONE SOILS

Denbigh and Flint

		Llanelwy D 40		*Gwaunysgor* 49		*Gwernaffield* 44		*Caerwys* 13		*Nannerch* 15		*Lixum* 28		*Cwm* 35	
		Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil
	Fine Gravel ...	3·53	3·77	4·43	—	9·22	13·55	7·52	9·40	4·09	5·89	5·31	5·65	5·57	12·45
	Coarse Sand ...	29·03	27·38	16·69	—	8·88	13·02	19·68	19·04	27·72	31·98	26·44	27·27	12·34	14·40
	Fine Sand ...	20·37	20·52	24·55	—	21·05	20·87	14·76	16·16	16·11	17·90	18·14	19·63	9·80	9·83
	Silt	15·57	9·80	11·47	—	20·07	19·91	13·83	17·25	14·98	12·04	15·19	15·17	12·90	12·46
	Fine Silt ...	12·80	17·52	21·16	—	22·09	17·98	22·35	19·55	18·76	15·83	18·60	18·16	33·16	29·12
	Clay	8·76	14·26	7·96	—	3·89	4·43	7·86	8·69	5·58	5·33	5·11	5·91	11·59	12·66
	Moisture ...	·68	·50	2·10	—	3·82	1·58	1·86	·90	4·70	3·48	1·90	·54	2·52	2·14
	Organic Matter ...	5·58	4·24	6·72	—	8·16	5·78	8·40	5·84	6·56	5·08	7·02	5·44	8·46	5·64
	Calcium Carbonate ...	·51	·23	4·54	—	2·04	1·02	·59	·26	1·86	·42	·16	·26	1·15	1·35
	Nitrogen ...	·180	—	·207	—	·237	—	·288	—	·193	—	·238	—	·220	—
48 hours digestion with HCl	Potash (K_2O) ...	·540	—	·690	—	·731	—	·595	—	·500	—	·476	—	·612	—
	Phosphoric Acid (P_2O_5) ...	·068	—	·092	—	·104	—	·154	—	·121	—	·144	—	·109	—
	Calcium Oxide (CaO) ...	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Magnesium Oxide (MgO) ...	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Insoluble ...	73·2	—	76·65	—	73·98	—	75·76	—	77·75	—	80·38	—	72·34	—
Soluble in 1 % Citric	Potash (K_2O) ...	—	—	·017	—	·024	—	·032	—	·024	—	·034	—	·024	—
	Phosphoric Acid (P_2O_5) ...	—	—	·017	—	·005	—	·010	—	·006	—	·018	—	·005	—

possible to make a more satisfactory classification. From the nature of the processes by which these soils have been formed one cannot be surprised at their extreme variability.

It is rather noteworthy that a large number of samples contain little or no calcium carbonate. This is the more surprising in view of the fact that the corresponding rainfalls are not excessive: the average annual rainfall at Lligwy in Anglesey for example is not more than about 35″, while that on the Orme at Llandudno is not more than 30″.

2. *Millstone Grit Soils.*

These soils are always found adjacent to the limestone and apart from the deficiency or absence of calcium carbonate they resemble to a large extent the soils of that formation. They present no features of special interest. We may remark that as in the case of the limestone so in the case of this formation a large portion consists of hill pasture. A single sample from the so-called Gwespyr Sandstone seems identical with the Millstone Grit type. Generally these soils are not of the highest quality, but this may be attributed to a large extent to their situation at comparatively high altitudes.

3. *West Carnarvonshire Light Loam.*

This type of soil occupies a considerable area in the west of Carnarvonshire and is roughly bounded by a line running from Pwllheli to Nevin. Within this area the soil generally belongs to this type. There are, however, patches of heavier soil and also of sand and gravel. In the centre of the district there is a broad stretch of wet lowland which was probably in former times an arm of the sea and is now mainly peat and sand. West of Pwllheli there is a tract of estuarine alluvium. The agriculture of the area is of a fairly good character. In normal times the land is farmed on the usual rotation of North Wales which includes a period of three, four or more years under grass. The rearing of store cattle is the mainstay of west Carnarvonshire farming. The principal corn crop is, as elsewhere, oats, but there is also a considerable area under barley, in which it differs markedly from Anglesey. The two areas are in many other respects comparable, but the Anglesey soils would appear to be rather more fertile.

Although the soils of this type are generally the result of the northern glaciation, there is generally an admixture of local material. Microscopical examination of the sand fractions shows grains of rounded quartz together with fragments of shaly and igneous rocks. If the

		Millstone Grit														Gwespyr Sandstone	
		Nerquis 16		*Hope* 20		*Halkyn* 23		*Halkyn* 24		*Gronant* 48		*Treiddyn* 50		*Rhydattalog* 51		*Talacre* 9	
		Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
	Fine Gravel ...	5·36	7·16	3·09	6·34	3·78	8·12	4·78	5·11	7·15	12·92	9·27	13·95	4·78	9·88	1·68	—
	Coarse Sand ...	16·51	13·61	24·73	24·14	14·35	15·16	21·14	20·71	32·24	31·55	19·73	20·36	17·46	22·47	25·43	—
	Fine Sand ...	25·66	20·35	24·59	23·00	22·03	22·74	18·54	18·94	20·09	19·58	23·40	25·40	23·25	23·54	21·15	—
	Silt	16·06	20·96	16·72	18·09	12·41	17·59	12·80	16·06	17·04	21·38	14·32	18·23	12·54	16·25	13·38	—
	Fine Silt ...	17·17	15·19	14·96	13·24	21·78	17·63	21·26	20·16	8·99	3·69	15·97	8·19	12·34	11·02	16·27	—
	Clay ...	5·90	13·67	6·42	6·78	5·15	8·20	5·23	6·81	4·35	3·40	4·56	5·29	3·21	7·39	5·96	—
	Moisture ...	2·08	1·62	·90	·58	7·34	1·92	3·08	2·24	3·12	1·18	2·06	1·32	7·40	1·72	4·06	—
	Organic Matter	9·08	4·94	6·32	4·36	9·70	5·60	8·54	6·26	5·78	3·96	9·18	5·38	15·38	5·56	10·44	—
	Calcium Carbonate ...	0·00	0·00	0·00	0·00	0·00	0·00	0·85	0·80	0·00	0·00	0·00	0·00	0·00	0·00	·44	—
	Nitrogen ...	·277	—	·225	—	·330	—	·287	—	·196	—	·291	—	·393	—	·338	—
48 hours digestion with HCl	Potash (K_2O) ...	·490	—	·483	—	·527	—	·551	—	·425	—	·350	—	·343	—	·473	—
	Phosphoric Acid (P_2O_5) ...	·097	—	·195	—	·141	—	·153	—	·092	—	·122	—	·127	—	·176	—
	Calcium Oxide (CaO) ...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Magnesium Oxide (MgO)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Insoluble ...	83·28	—	85·04	—	79·49	—	77·77	—	84·29	—	84·28	—	82·38	—	76·26	—
Soluble in 1 % Citric	Potash (K_2O) ...	·041	—	·031	—	·040	—	·039	—	·012	—	·015	—	·014	—	0·051	—
	Phosphoric Acid (P_2O_5) ...	·009	—	·012	—	·007	—	·009	—	·005	—	·012	—	·009	—	0·009	—

West Carnarvonshire Light Loam

		Glanrhyd C 3		*Aberdaron* C 5		*Bottwnog* C 19		*Tudweiliog* C 55		*Tudweiliog* C 59		*Bodvel Hall* C 60		*Llithfaen* C 62		*Mynytho* C 63		*Bottwnog* C 64	
		Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil
	Fine Gravel ...	3·23	5·10	4·70	5·00	7·17	7·59	1·26	1·35	6·97	—	4·80	3·28	9·13	8·39	5·98	4·73	7·34	10·95
	Coarse Sand ...	22·51	18·02	22·00	23·96	19·77	22·19	11·13	8·18	15·66	—	16·92	18·95	9·28	9·25	12·45	14·31	19·54	21·11
	Fine Sand ...	24·24	24·00	24·99	23·20	22·02	20·68	35·40	42·06	27·07	—	32·05	36·22	25·63	24·14	29·15	26·47	25·89	27·20
	Silt	10·52	13·89	15·29	13·94	13·21	18·30	20·00	25·90	12·87	—	14·64	15·65	15·57	17·63	16·33	24·42	12·43	16·40
	Fine Silt ...	14·23	19·04	14·66	17·54	21·32	12·48	14·26	8·50	19·36	—	17·88	14·21	20·58	21·44	19·83	16·15	17·23	9·18
	Clay	2·81	5·34	5·40	5·93	5·67	9·10	4·52	6·01	4·49	—	3·24	3·01	3·89	3·85	2·31	3·72	4·31	3·87
	Moisture ...	7·00	5·14	3·84	2·60	1·30	1·48	2·00	2·12	2·44	—	2·14	1·74	3·70	3·48	2·56	1·08	2·64	2·30
	Organic Matter	8·18	5·50	7·14	5·54	7·22	4·08	6·66	4·78	9·74	—	9·36	5·22	13·16	11·48	9·72	7·96	9·34	6·28
	Calcium Carbonate ...	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	—	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	Nitrogen ...	·325	—	·251	—	·257	—	·204	—	·343	—	·324	—	·418	—	—	—	·324	—
48 hours digestion with HCl	Potash (K_2O) ...	·420	—	·417	—	·456	—	·415	—	·472	—	·411	—	·408	—	·306	—	·595	—
	Phosphoric Acid (P_2O_5) ...	·207	—	·185	—	·140	—	·171	—	·186	—	·162	—	·182	—	·200	—	·205	—
	Calcium Oxide (CaO) ...	·21	—	·22	—	—	—	—	—	—	—	—	—	·23	—	·17	—	—	—
	Magnesium Oxide (MgO)	·48	—	·28	—	—	—	—	—	—	—	—	—	·04	—	·13	—	—	—
	Insoluble ...	73·1	—	79·1	—	78·6	—	81·6	—	76·5	—	79·4	—	71·2	—	77·2	—	79·6	—
Soluble in 1 % Citric	Potash (K_2O) ...	·031	—	·019	—	—	—	—	—	—	—	—	—	·029	—	·036	—	—	—
	Phosphoric Acid (P_2O_5) ...	·020	—	·026	—	—	—	·015	—	·018	—	·009	—	·010	—	·024	—	·011	—

	Meillionydd C 66		Meillionydd C 67		Penmaen C 74		Bodvel Hall C 75		Bodgadle C 76		Llaniestyn C 79		Aberdaron C 80	
	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil
Fine Gravel ...	5·53	8·80	6·42	11·38	5·51	6·96	6·01	6·54	4·38	5·73	11·60	9·14	11·65	—
Coarse Sand ...	16·43	21·87	18·96	22·04	24·84	19·25	14·87	15·83	15·84	17·03	12·37	10·27	8·83	—
Fine Sand ...	24·64	27·02	22·44	22·60	24·20	29·33	24·72	22·70	28·24	26·40	23·65	27·50	19·70	—
Silt	16·85	19·59	16·05	21·08	15·48	18·70	16·25	17·11	14·10	19·18	13·07	14·99	17·02	—
Fine Silt ...	15·61	8·25	16·43	6·90	14·46	12·27	20·06	22·54	15·97	12·23	16·84	17·17	20·73	—
Clay	4·14	6·19	3·40	3·82	2·10	3·25	3·54	4·18	4·12	5·44	6·52	9·42	6·97	—
Moisture ...	3·30	1·64	2·72	2·38	3·40	2·24	3·64	2·24	3·48	2·36	1·90	1·48	2·28	—
Organic Matter	10·26	5·42	10·36	6·20	9·08	4·42	7·92	6·56	10·50	8·02	9·50	7·44	9·90	—
Calcium Carbonate ...	Nil	Nil	Nil	Nil	Nil	Nil	—	—	—	—	—	—	—	—
Nitrogen ...	·348	—	·364	—	·318	—	·284	—	·334	—	—	—	—	—
48 hours digestion with HCl: Potash (K_2O) ...	·466	—	·448	—	·429	—	·490	—	·500	—	—	—	—	—
48 hours digestion with HCl: Phosphoric Acid (P_2O_5) ...	·108	—	·139	—	·083	—	·091	—	·132	—	—	—	—	—
48 hours digestion with HCl: Calcium Oxide (CaO) ...	—	—	—	—	—	—	—	—	—	—	—	—	—	—
48 hours digestion with HCl: Magnesium Oxide (MgO)	—	—	—	—	—	—	—	—	—	—	—	—	—	—
48 hours digestion with HCl: Insoluble ...	77·3	—	75·2	—	76·7	—	77·1	—	76·9	—	—	—	—	—
Soluble in 1 % Citric: Potash (K_2O) ...	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Soluble in 1 % Citric: Phosphoric Acid (P_2O_5) ...	—	—	—	—	—	—	—	—		—	—	—	—	—

rounded quartz grains were subtracted the resultant soil would probably be similar to the Carnarvonshire Stony Loam. These soils are, however, by no means so stony as that type.

The subsoils are generally similar in character to the soils, but it is quite common to encounter beds of gravel or clay at lower levels. The drift geology has been well worked out by Jehu[1] from whose paper most of the information about the drifts of this district is derived. It is remarkable that the variety on the surface is not so great as might be expected from the vertical succession in exposed cuttings. Clay soils are not common; yet in most vertical successions, strata of clay are recorded. The clay of the geologist is however not always the same as the clay of the soil chemist. Some of the strata described as clay in geological papers are not clays at all in the strict sense of the word, but rather loams.

These soils are generally of a dark brown colour and of a good open texture. They do not seem to present any special difficulties in cultivation. The figures given in the tables are the average of 16 analyses. As will be seen, the various fractions are fairly well balanced and the complete analyses recall to some extent the figures for the Anglesey Medium Loam. The dividing line between these soils and the sands and gravels is purely arbitrary. We have included in this class all soils with less than 50 per cent. of sand.

The west Carnarvonshire soils as a whole may be considered as local débris with a greater or less admixture of marine drift from the north. If this marine drift were absent the soils would approximate closely to the Carnarvonshire Stony Loam. On the other hand where the external drift predominates, the soil approaches to the ordinary type of glacial sand or gravel. The present type is therefore intermediate or mixed.

4. *Glacial Sands and Gravels.*

These soils are found in the eastern portion of the area and also, to a less extent, in west Carnarvonshire. It is a matter of considerable difficulty to define accurately the boundaries of these soils as they are mixed in almost inextricable confusion with boulder clay and, in the Vale of Clwyd, with alluvial deposits. We may, however, note a long belt running along the eastern side of the latter area and sporadic deposits along the valleys of the Alyn and the Dee. In the

[1] "The Glacial Deposits of West Carnarvonshire." T. J. Jehu. *Trans. Roy. Soc. Edin.* **42**, Part I, No. 2.

SANDS AND GRAVELS, WEST CARNARVONSHIRE

		Nevin C 2		Ty Engan C 6		Barrach C 7		Mathan Uchaf C 56		Madryn C 57		Bodvel Hall C 58		Llanbedrog C 68		Llanbedrog C 69	
		Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil
	Fine Gravel ...	7·99	7·84	4·44	5·83	5·55	2·85	2·83	1·16	6·75	8·78	4·12	6·10	11·24	5·19	5·38	6·34
	Coarse Sand ...	41·12	42·06	19·85	19·87	24·64	19·37	65·86	68·98	35·85	54·70	31·85	33·63	42·93	8·73	29·19	23·75
	Fine Sand ...	20·01	21·03	29·09	32·48	30·00	41·34	14·40	15·58	22·97	14·49	30·64	33·33	17·27	40·86	22·48	22·64
	Silt ...	9·19	9·05	12·75	21·12	9·01	10·05	5·62	6·71	11·90	11·65	10·45	15·87	11·70	23·53	17·73	21·36
	Fine Silt ...	7·92	8·05	16·73	15·17	15·65	12·48	2·67	2·30	9·40	4·20	11·32	2·21	5·08	5·28	9·05	11·38
	Clay ...	·78	·76	3·04	2·63	3·56	6·44	1·23	1·43	2·57	1·67	2·12	2·23	2·43	6·19	3·42	5·16
	Moisture ...	3·64	4·22	4·30	3·48	2·88	1·18	1·08	1·02	2·02	1·50	1·88	1·30	1·48	1·96	1·92	1·58
	Organic Matter	7·70	4·64	7·96	7·18	6·52	3·34	5·50	3·46	7·50	1·58	6·36	4·08	10·70	5·90	7·48	7·26
	Calcium Carbonate ...	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	Nitrogen ...	·245	—	·279	—	·199	—	·142	—	·256	—	·261	—	·325	—	—	—
48 hours digestion with HCl	Potash (K_2O) ...	300	—	·414	—	·426	—	·198	—	·340	—	·357	—	·313	—	·612	—
	Phosphoric Acid (P_2O_5) ...	·147	—	·141	—	·055	—	·100	—	·121	—	·202		·179	—	·139	—
	Calcium Oxide (CaO) ...	·20	—	·26	—	·22	—	·07	—	—	—	—	—	—	—	—	—
	Magnesium Oxide (MgO) ...	·30	—	·15	—	·08	—	—	—	—	—	—	—	—	—	—	—
	Insoluble ...	81·3	—	76·7	—	75·4	—	89·1	—	81·7	—	80·1	—	84·2	—	72·3	—
Soluble in 1 % Citric	Potash (K_2O) ...	·016	—	·019	—	·020	—	·029	—	—	—	—	—	—	—	—	—
	Phosphoric Acid (P_2O_5) ...	·009	—	·007	—	·006	—	·015	—	·011	—	·018	—	·004	—	·006	—

		West Carnarvonshire						Transition Type					
		Penrhos C 71		*Bodgadle* C 77		*Abersoch** C 11		*Rhual* 25		*Mynydd Mostyn* 8		*Nerquis* F 17	
		Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil
	Fine Gravel ...	8·21	3·13	14·02	19·87	5·75	6·61	10·41	12·75	4·89	8·46	6·99	7·14
	Coarse Sand ...	36·42	37·45	31·70	31·13	63·34	66·01	25·24	24·89	27·90	24·81	27·32	26·52
	Fine Sand ...	24·04	22·81	22·95	19·71	9·67	9·75	16·81	20·28	21·67	21·40	18·38	20·79
	Silt ...	12·17	13·53	11·64	13·19	6·69	8·15	11·22	11·30	14·44	17·98	12·73	12·79
	Fine Silt ...	6·90	12·01	9·58	6·37	6·61	3·78	16·12	14·96	11·87	10·44	16·23	16·05
	Clay	2·82	1·23	2·08	2·83	1·18	1·61	2·80	3·52	6·79	7·04	4·50	6·14
	Moisture ...	2·16	1·40	1·72	1·16	1·46	·82	2·20	1·64	1·86	1·12	1·58	1·10
	Organic Matter	7·34	5·62	7·14	5·30	5·52	2·70	10·44	7·32	7·26	5·36	9·12	6·16
	Calcium Carbonate ...	Nil	Nil	Nil	Nil	Nil	Nil	2·16	·91	·47	·48	1·51	·59
	Nitrogen ...	—	—	—	—	·188	—	·309	—	·238	—	·276	—
48 hours digestion with HCl	Potash (K_2O) ...	·286	—	—	—	·340	—	·500	—	·476	—	·459	—
48 hours digestion with HCl	Phosphoric Acid (P_2O_5) ...	·125	—	—	—	·122	—	·215	—	·133	—	·168	—
48 hours digestion with HCl	Calcium Oxide (CaO) ...	—	—	—	—	·32	—	—	—	—	—	—	—
48 hours digestion with HCl	Magnesium Oxide (MgO) ...	—	—	—	—	·20	—	—	—	—	—	—	—
48 hours digestion with HCl	Insoluble ...	82·8	—	—	—	85·5	—	74·28	—	81·01	—	78·37	—
Soluble in 1% Citric	Potash (K_2O) ...	—	—	—	—	·027	—	·033	—	·032	—	·044	—
Soluble in 1% Citric	Phosphoric Acid (P_2O_5) ...	—	—	·039	—	·066	—	·020	—	·008	—	·022	—

* This soil was included in the former paper as a wind blown sand. Further examination has shown that this was an error and that it should be classified as a glacial sand.

SANDS AND GRAVELS, EASTERN

	Hawarden 19		Nannerch 27		Soughton 29		Hanmer Whitchurch 38		Penley 39		Bettisfield 40		Near Whitchurch 41		Llandyrnog D 11		Gresford D 14		Wrexham D 47		Wrexham D 49	
	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil
Fine Gravel ...	3·80	7·16	6·98	6·37	5·53	5·85	3·83	2·40	1·09	·36	1·77	2·29	1·26	2·49	2·10	4·07	2·20	3·29	8·04	7·44	4·49	3·98
Coarse Sand ...	51·45	52·90	49·16	49·84	39·51	42·03	53·64	60·60	39·18	43·18	63·38	66·38	52·21	53·25	53·89	59·41	37·62	42·56	29·60	31·27	35·98	38·18
Fine Sand ...	13·81	13·16	13·76	15·33	21·68	20·99	17·57	17·85	23·64	23·23	16·00	18·78	17·95	18·70	12·46	12·76	21·78	24·10	21·21	18·85	23·84	23·72
Silt	7·40	8·91	8·15	10·95	9·06	11·18	6·48	4·58	10·40	10·79	5·80	4·76	7·28	9·30	7·19	8·54	9·95	9·01	10·59	12·42	8·23	7·97
Fine Silt ...	9·70	7·83	10·77	7·96	12·31	11·22	6·37	6·06	11·99	9·27	5·69	3·84	8·57	4·93	14·63	13·04	11·47	8·61	14·07	15·76	12·23	13·42
Clay	3·61	4·03	2·28	1·89	3·04	2·94	6·03	4·53	6·78	8·37	1·81	1·10	4·66	6·77	2·67	2·86	2·73	2·27	2·64	4·22	2·04	3·40
Moisture ...	1·34	·54	·68	·62	1·60	·66	0·92	0·70	1·52	0·86	1·20	·60	1·90	1·00	1·12	1·20	2·58	2·06	1·98	1·62	1·78	—
Organic Matter	7·56	4·26	7·38	4·80	6·54	3·90	3·28	1·90	4·64	2·68	3·40	1·80	1·70	3·16	4·62	3·22	10·26	7·34	9·62	6·60	9·96	—
Calcium Carbonate ...	0·19	0·04	0·28	0·48	0·00	0·00	0·00	0·00	0·00	0·00	·17	0·00	0·00	0·00	·42	·16	·14	0·00	·32	—	—	—
Nitrogen ...	·230	—	·255	—	·210	—	·125	—	·161	—	·136	—	·167		·176	—	·408	—	·451	—	·252	—
48 hours digestion with HCl: Potash (K_2O) ...	·343	—	·493	—	·320	—	·418	—	·568	—	·282	—	·350	—	·448	—	·320	—	·408	—	·382	—
Phosphoric Acid (P_2O_5) ...	·196	—	·140	—	·130	—	·104	—	·088	—	·084	—	·106	—	·071	—	·108	—	·197	—	·156	—
Calcium Oxide (CaO) ...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	·41	—	·32	—	—	—	—	—
Magnesium Oxide (MgO)...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	·18	—	·20	—	—	—	—	—
Insoluble ...	84·47	—	81·89	—	85·04	—	89·05	—	85·55	—	90·72	—	87·55	—	85·2	—	77·4	—	78·38	—	80·0	—
Soluble in 1 % Citric: Potash (K_2O) ...	·016	—	·049	—	·018	—	·009	—	·013	—	·014	—	·018	—	·016	—	·013	—	·015	—	·019	—
Phosphoric Acid (P_2O_5) ...	·038	—	·023	—	·019	—	·027	—	·010	—	·013	—	·018	—	·015	—	·005	—	·015	—	·033	—

parts of Flintshire bordering on the Dee sandy deposits occur in no very well defined order mingled with boulder clay.

Here again there seems to be no very characteristic type of farming. In the eastern portion, however, there are, here and there, market gardens. While in the Vale of Clwyd very good farming is found on the sands, one frequently finds elsewhere rather poor land, particularly along banks where springs occur as in the valleys of the Alyn and the Dee. The drainage of such soils presents difficulties as there are often beds of clay to complicate matters. Were such drainage carried out it is probable that these soils might be useful for market gardening.

In the tables we have separated the soils of the eastern area from those of west Carnarvonshire. A comparison of the figures shows that the eastern soils are somewhat more sandy than the others. It will be seen also that they contain more potash and slightly less organic matter. Further, an inspection of the individual analyses will show that the eastern soils occasionally contain calcium carbonate, while those of west Carnarvonshire never contain any.

Apart from this, however, the two sets of soils are very similar and, although they are averaged separately, they must be considered as one series. The difference in the amount of organic matter is less than one would have expected, considering that west Carnarvonshire is wetter than the eastern regions. As stated above, the dividing line between the glacial sands and the other glacial types is purely arbitrary. We have noted three soils in Flintshire which appear to form a transition type between this type and the heavier drift soils of that district.

5. *Heavy Drift Soils.*

Two types of heavy drift soils may be recognised, namely the eastern type in eastern Flintshire and the English border, and the Vale of Clwyd type which occurs in the vale and the adjoining coastal districts of Flintshire and Denbighshire. There would also appear to be a subtype of the eastern type in the detached portion of Flintshire. There are also isolated patches of heavy drift soil in west Carnarvonshire.

(*a*) *Eastern type.*

The soils of this type are best described as heavy loams and are of a brownish colour. In the lowland districts they are of fair fertility and carry good crops of wheat. The grass land is often of high quality. The soils of the detached portion of Flintshire are rather heavier and may be described as clays. As the English border is approached the

HEAVIER DRIFT SOILS, EASTERN MAIN TYPE

		Leeswood 18		*Broughton* 21		*Aston* 22		*Northop* 30		*Ewloe Green* 31		*Pen-y-ffordd* 32	
		Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil
	Fine Gravel ...	2·39	3·79	1·52	2·75	2·74	2·06	3·81	3·32	2·22	2·83	3·99	4·91
	Coarse Sand ...	28·66	29·68	21·24	20·48	27·25	24·81	27·59	25·27	16·71	12·65	25·58	26·46
	Fine Sand ...	18·81	17·61	22·37	22·49	21·42	18·46	19·18	16·52	20·70	25·43	21·92	19·79
	Silt	10·42	12·87	14·09	13·62	11·62	12·93	12·23	11·68	16·36	18·89	11·93	11·38
	Fine Silt ...	18·64	16·49	18·68	23·40	16·61	16·13	15·46	16·01	16·85	15·66	15·72	14·46
	Clay ...	8·01	13·22	9·11	12·79	9·12	16·17	13·58	21·08	14·44	15·49	8·90	14·28
	Moisture ...	1·48	1·04	1·78	1·66	1·82	1·10	1·90	2·04	4·38	3·12	2·20	1·76
	Organic Matter	8·32	4·24	8·66	5·62	6·86	4·48	6·02	4·40	7·60	5·10	7·52	4·50
	Calcium Carbonate ...	0·00	0·11	0·00	0·00	0·00	0·00	0·39	0·15	1·60	0·47	0·97	0·39
	Nitrogen ...	·259	—	·267	—	·180	—	·200	—	·196	—	·207	—
48 hours digestion with HCl	Potash (K_2O) ...	·473	—	·598		·500	—	·537	--	·517	—	·479	—
	Phosphoric Acid (P_2O_5) ...	·144	-	·141	—	·125	—	·092	—	·123	—	·121	—
	Calcium Oxide (CaO)												
	Magnesium Oxide (MgO)												
	Insoluble ...	80·27	—	78·26	--	81·32	—	80·74	—	76·04	—	79·66	—
Soluble in 1% Citric	Potash (K_2O) ...	·040	—	·033	—	·028	—	·095	—	·037	—	·024	—
	Phosphoric Acid (P_2O_5) .	·018	—	·013	—	·022	—	·009	—	·012	—	·016	—

MAELOR SUB-TYPE

		Holt D 12		*Dirty Mile* 33		*Bagillt* 47		*Worthenbury* 37		*Bangor on Dee* 42		*Overton* 43	
		Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil
	Fine Gravel ...	1·09	·75	2·72	3·86	5·04	9·43	1·81	1·60	2·69	2·55	2·12	3·33
	Coarse Sand . .	17·38	19·97	27·54	30·86	32·08	32·40	13·17	10·01	7·93	11·74	10·14	8·03
	Fine Sand ...	14·36	15·18	21·84	20·35	18·90	19·69	10·12	9·11	12·92	14·84	12·26	6·76
	Silt	14·31	11·39	12·31	11·44	13·35	11·55	13·06	12·22	13·05	13·17	13·72	14·26
	Fine Silt ...	26·30	24·55	14·52	13·06	12·21	10·77	27·45	28·25	28·46	25·55	35·25	35·15
	Clay	11·70	16·56	7·86	15·29	7·32	9·85	16·47	27·06	15·87	22·47	12·02	23·22
	Moisture ...	3·06	2·46	1·38	1·00	2·44	1·44	4·84	2·84	5·40	1·80	3·04	1·40
	Organic Matter	10·82	5·32	7·72	4·02	7·30	4·50	9·86	5·54	10·12	5·88	11·36	6·26
	Calcium Carbonate ...	Nil	Nil	0·67	0·26	0·00	0·00	0·00	0·24	0·00	0·00	0·00	0·00
	Nitrogen ...	·366	—	·217	—	·207	—	·328	—	·322	—	·330	—
48 hours digestion with HCl	Potash (K_2O) ...	·601	—	·435	—	·544	—	1·05	—	·819	—	·993	—
	Phosphoric Acid (P_2O_5) ...	·124	—	·101	—	·134	—	·184	—	·107	—	·136	—
	Calcium Oxide (CaO) ...	·28	—	—	—	—	—	—	—	—	—	—	—
	Magnesium Oxide (MgO)	·26	—	—	—	—	—	—	—	—	—	—	—
	Insoluble ...	73·6	—	82·35	—	79·89	—	68·14	—	68·85	—	69·33	—
Soluble in 1% Citric	Potash (K_2O) ...	·008	—	·017	—	·015	—	·024	—	·014	—	·020	—
	Phosphoric Acid (P_2O_5) ...	·014	—	·017	—	·012	—	·037	—	·013	—	·014	—

farming approximates more and more to the Cheshire and north Shropshire type. These soils are in fact not so characteristic of Wales as some of the other soils studied. Although of external origin, they appear to be generally composed of material from the Coal Measures. The heavier soils of the detached portion of Flintshire have the reddish appearance of soils composed of Triassic material.

They are not markedly stony, although often described as Boulder Clay. The subsoils are rather stiff and compact. The figures given for the main type are the average of nine analyses. It will be observed that they do not indicate a very stiff soil but rather a heavy loam, which, in the wetter districts, may present all the difficulties of a clay. The proportion of clay in the subsoils is considerably higher than in the soils. The figures for potash are not particularly high. Three soils from the detached portion of Flintshire (Maelor) are separately averaged. They are considerably heavier and contain high proportions of potash. While the eastern type is generally lacking in calcium carbonate, there are a few samples which contain small amounts of this constituent.

The heavier drift soils of west Carnarvonshire are relatively of such little importance that they have not been separately averaged, but the results are shown in the table for heavier drift soils.

(*b*) *Vale of Clwyd type.*

The soil is somewhat stiffer than the other type and is the heaviest soil encountered in North Wales so far. It can, in fact, be described as a clay soil. It is of a reddish colour except where the natural colour is masked by much organic matter. It is probably mainly composed of Triassic material[1].

The Vale of Clwyd has a high reputation for fertility which may be attributed partly to the climate, which is distinctly drier than the other districts, and partly to the general level character of the ground. The Vale is certainly earlier than the adjoining uplands and has a general

[1] It has been suggested that this clay is a deposit carried from the north and deposited from melting icebergs. However this may be, I have noted the same deposit in Carnarvonshire and it has been found by others in Anglesey. In the former county, at the College farm, Aber near Bangor, there is a deposit of clay about 18 inches or more from the surface in all respects similar to the Vale of Clwyd type. Dr G. H. Bryan, F.R.S., has distinguished fragments of foraminifera in this clay but was not able to name them. Foraminifera have also been found in the Clwydian clay. The clay in the Bangor and Aber district is everywhere obscured by local drift. I have also noticed it in a cutting near Penmaenmawr. G. W. R.

See "Drifts of the Vale of Clwyd, etc." T. McKenny Hughes, *Q.J.G.S.*, Feb., 1887.

Heavier Drift Soils.

VALE OF CLWYD TYPE

		Prestatyn F 5		*Dyserth* F 4		*Rhuddlan* F 36		*Bodelwyddan* F 54		*Lleweni* D 19		*Ruthin* D 25	
		Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil
	Fine Gravel ...	2·71	3·90	2·42	1·83	2·10	3·75	2·86	3·27	2·14	1·34	1·35	1·58
	Coarse Sand ...	33·61	25·75	29·06	25·07	27·82	23·21	18·45	16·74	12·31	8·98	29·27	21·53
	Fine Sand ...	21·43	16·80	20·62	17·75	18·51	19·18	14·48	16·83	10·84	8·90	18·74	12·72
	Silt	10·91	11·70	11·14	10·84	11·22	12·30	13·47	11·68	12·48	11·25	11·61	10·32
	Fine Silt ...	13·25	14·92	11·41	16·08	17·10	17·47	21·54	22·13	34·44	29·88	13·34	19·44
	Clay	7·85	17·91	9·20	18·19	8·24	15·69	15·10	22·07	14·89	27·02	8·57	12·34
	Moisture ...	1·20	1·94	1·34	1·50	2·26	1·52	3·10	2·48	2·82	3·22	2·92	1·16
	Organic Matter	6·04	4·50	6·78	5·06	7·50	4·78	7·00	5·00	8·54	5·32	13·24	5·90
	Calcium Carbonate ...	2·11	·77	2·33	1·30	2·14	·47	·54	·54	·60	1·00	·43	·60
	Nitrogen ...	·162	—	·197	–	·192	—	·176	—	·242	–	·460	—
48 hours digestion with HCl	Potash (K_2O) ...	·568	—	·541	-	·534	—	·782	—	·860	—	·612	—
	Phosphoric Acid (P_2O_5) ...	·099	—	·067	—	·114	—	·083	—	·137	—	·091	—
	Calcium Oxide (CaO) ...	—	—	—	—	—	—	—	—	—	—	—	—
	Magnesium Oxide (MgO)	—	—	—	-	—	-	—	—	—	—	—	—
	Insoluble ...	83·1	--	78·37	—	78·51	—	76·35	—	73·7	—	70·3	—
Soluble in 1 % Citric	Potash (K_2O) ...	·039	—	·024	—	·021	—	·021	—	·021	—	—	—
	Phosphoric Acid (P_2O_5) ...	·008	—	·012	—	·007	—	·006	—	·012	—	—	—

		Rhyl F 1		*St Asaph* F 11		*Ty'n y Coed* D 6		WEST CARNARVONSHIRE *Hirwaen, Aberdaron* C 4		WEST CARNARVONSHIRE *Trefollwyn, Bottwnog* C 8	
		Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil
	Fine Gravel ...	1·18	2·10	·92	2·02	1·78	2·38	2·58	3·31	1·08	·97
	Coarse Sand ...	26·70	24·94	17·67	17·75	22·07	21·18	19·23	15·66	7·05	4·30
	Fine Sand ...	18·18	16·82	20·25	20·30	17·36	16·48	19·14	16·08	20·08	19·02
	Silt	12·04	11·60	16·96	15·75	11·90	11·14	12·84	10·70	22·22	21·10
	Fine Silt ..	19·13	18·91	20·11	19·19	18·94	19·83	21·18	20·53	24·85	23·92
	Clay	2·12	1·81	1·98	1·38	3·98	4·86	8·06	17·31	8·47	17·99
	Moisture ...	5·28	3·54	7·66	5·18	6·02	4·56	5·24	7·34	3·44	4·68
	Organic Matter	·98	1·08	Nil	Nil	·20	·06	7·18	4·86	8·80	5·01
	Calcium Carbonate ..	—	—	—	—	—	—	Nil	Nil	Nil	Nil
	Nitrogen ...	·147	--	·200	—	·193	—	·204	—	·259	—
48 hours digestion with HCl	Potash (K_2O) ...	·561	—	·632	-	·734	—	·578	—	·483	—
	Phosphoric Acid (P_2O_5)	·088	--	·078	-	·096	—	·103	—	·059	--
	Calcium Oxide (CaO) ...	—	—	—	—	·54	—	·31	—	·36	—
	Magnesium Oxide (MgO)	—	—	—	—	·54	—	·08	—	·42	—
	Insoluble ...	82·30	—	79·50	—	78·8	—	70·9	—	69·2	—
Soluble in 1 % Citric	Potash (K_2O) ...	·016	—	·036	—	·018	—	·008	—	·019	—
	Phosphoric Acid (P_2O_5) ...	·012	—	·008	--	·023		·005	—	·009	—

appearance of prosperity which suggests Shropshire or Cheshire rather than Wales. Good crops of wheat and beans are grown and the grass is frequently of excellent quality. The type of rotation is not greatly different from that in other parts of the area, but there is probably a greater proportion of permanent grass land than elsewhere, where much of the grass land comes at some period under the plough.

The results are assembled in the general table, which is the average of nine analyses. It will be seen that there is a fair but not large proportion of clay, although some soils greatly exceed the average.

There is usually a small proportion of calcium carbonate. In this respect the Clwyd soils differ from most of the soils of N. Wales and from the other heavy drift soils, which are deficient in this constituent. Potash is high, as might be expected, and phosphoric acid is somewhat low. It should be added that the subsoil is generally very stiff and, at great depths, has a bluish colour owing to the presence of ferrous iron compounds.

The proportions of organic matter are low considering that the soils are heavy. We may attribute this mainly to the climate and indirectly to the presence of small quantities of calcium carbonate which, however, would speedily be washed out if the rainfall were as high as that of the surrounding uplands. Of all the types studied this is the lowest in phosphoric acid. The figures for available phosphoric acid are also low. In view of the well-known fertility of the Vale, it would seem that we must take careful account of the climate in judging as to what are insufficient proportions of soil constituents. From a mere inspection of the analytical figures it would seem that these soils are suffering from phosphorus starvation, yet the facts in practice show that this is extremely unlikely.

6. *Alluvial Soils.*

Considerable stretches of river alluvium are found in the Vale of Clwyd and form rather stiff soils. Estuarine alluvium is found at the mouth of the Clwyd near Rhyl and in the reclaimed portions of the Dee estuary. These alluvia must be regarded as distinct sub-types.

(*a*) *Fluviatile alluvia.*

A tract of this type occurs in the Vale of Clwyd between Denbigh and Ruthin. It is a light grey soil and often very heavy. Much of it is badly drained and is in consequence uncultivated. Smaller patches of alluvium are also found along the banks of the Alyn and the Dee. The single sample, from Lleweni near Denbigh, typical of that area, is

remarkable for its high proportion of fine silt. As far as we know, the figures both for soil and subsoil are the highest recorded for this fraction.

(b) *Estuarine alluvia.*

These soils occur, as was mentioned, in two localities. The Clwyd estuary has a much heavier alluvium than the Dee. In the former area, owing to bad drainage, much of the land is in a rough state, but higher up the vale the cultivation is better. The Dee alluvia form an area which has been reclaimed in comparatively recent years. The soils vary from loams to coarse sands. This area is fairly flat and easily worked. The agriculture is very varied. Both market gardening and ordinary farming exist side by side. Some of the soil is of considerable fertility, but this is probably the result of good farming.

In the case of the alluvial soils it is useless to give average figures as the variations are so great. The figures given will serve rather as examples of the variations encountered.

Alluvial Soils.

		Estuarine										Fluviatile	
		Rhuddlan 2		*Gwern Igron*		*Beeches Sandycroft* 6		*Sealand* 7 (*Queensferry*)		*Sealand* 34		*Lleweni*	
		Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil	Soil	Subsoil
	Fine Gravel ...	·04	·12	2·12	—	·40	·06	·11	·05	·16	·31	·69	·54
	Coarse Sand ...	·73	·24	3·49	—	1·45	2·12	·60	·25	5 51	3·50	·54	1·99
	Fine Sand ...	7·40	4·36	8·70	—	59·90	70·77	56·75	54·12	76·64	72·87	2·51	2·90
	Silt	22·08	20·89	25·90	—	11·36	7·66	11·22	12·88	2·27	4·01	9·26	12·69
	Fine Silt ...	32·86	34·04	37·32	—	11·51	8·44	12·78	11·22	2·67	4·99	57·59	50·82
	Clay	20·09	28·68	7·72	—	5·30	5·72	5·61	8·72	1·08	1·88	13·90	19·47
	Moisture ...	3·48	3·16	2·4	—	1·28	·36	4·24	3·08	·24	·46	2·74	3·10
	Organic Matter	10·92	5·44	11·8	—	6·42	2·42	5·58	4·82	5·44	6·74	9·52	6·94
	Calcium Carbonate ...	·14	·16	—	—	·37	·41	3·28	4·45	6·37	6·07	Nil	Nil
	Nitrogen ...	·374	—	—	—	·251	—	·172	—	·05	—	·302	—
	Potash (K_2O) ...	·537	—	—	—	·578	—	·578	—	·258	—	·982	—
48 hours digestion with HCl	Phosphoric Acid (P_2O_5) ...	·142	—	—	—	·115	—	·127	—	·085	—	·109	—
	Calcium Oxide (CaO) ...	—	—	—	—	—	—	—	—	—	—	·28	—
	Magnesium Oxide (MgO)	—	—	—	—	—	—	—	—	—	—	·58	—
	Insoluble ...	69·23	—	—	—	83·29	—	79·68	—	85·33	—	68·8	—
Soluble in 1% Citric	Potash (K_2O) ...	·058	—	—	—	·043	—	·026	—	·035	—	·012	—
	Phosphoric Acid (P_2O_5) ...	·017	—	—	—	·017	—	·017	—	·008	—	·010	—

Conclusion.

Many of the soils of this paper do not differ greatly from the soils of the adjoining parts of England and, since they are found in the regions of North Wales which have the lowest rainfall, they are not so typical as those described in the former paper. The Carboniferous and Millstone Grit soils are the only sedentary soils examined in the present paper. The sedentary soils of the older Palæozoic formations showed the rather remarkable fact that the coarsest fractions were not generally the richest in silica. While we have not made determinations of the composition of the fractions of the sedentary soils of this paper, yet it is obvious from inspection that they are exactly similar to the majority of soils hitherto examined elsewhere in that their coarsest fractions consist almost entirely of quartz sand. In fact the character of the coarse fractions forms a useful criterion for distinguishing between these soils and the older Palæozoic soils.

The general poverty in calcium carbonate is noteworthy. Even some of the soils derived from the Carboniferous Limestone are lacking in this soil constituent. This is rather remarkable when it is considered that some of the chalk soils examined by Hall and Russell in their survey of Kent, Surrey and Sussex are formed under rainfalls at least as great as that in some of the limestone districts of our area. The matter is at least worth investigation.

Even the very sandy soils contain reasonable quantities of potash This as was suggested elsewhere[1] is doubtless due to the presence of potash minerals in the finer fractions. Although the soils described above are considerably younger than the typical Palaeozoic soils, they differ to some extent from the soils of S.E. England whose particles have undergone numerous vicissitudes of weathering, sorting and deposition which have resulted in their soluble constituents being leached out.

It is a fortunate circumstance that the heaviest clays in North Wales occur under the driest climate. We are not prepared to say that this is quite fortuitous but the exact connection, if any, is not quite clear, and needs further consideration. Were clays as heavy as those of the Vale of Clwyd to occur in the wetter hill districts, it is difficult to see how they could be worked.

In conclusion, we would thank, besides those gentlemen mentioned in the earlier paper, Mr G. J. Williams, H.M. Inspector of Mines and Quarries, and Mr Edward Greenly, the author of the Anglesey Geological Survey,-for their kind counsel on geological questions.

[1] "Soils of North Wales." G. W. Robinson. *Journ. Bd. of Agric.* June, 1915.

(*Received April 22nd*, 1919.)

THE FUNGICIDAL PROPERTIES OF CERTAIN SPRAY-FLUIDS. II.

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Introductory

In a previous communication[1] we have adduced evidence to show that solutions of alkaline sulphides are probably of fungicidal value owing to the polysulphides which they contain.

This work has been continued along similar lines, and the following account gives the main results obtained during the past three years 1916–18. During the first two years a number of ammonium polysulphide solutions were made according to different methods, and the fungicidal value of each determined with a view of ascertaining whether a relationship existed between the polysulphide sulphur content and the fungicidal action of these solutions.

During this period evidence accumulated that the death-point of the mildew (*Sphaerotheca Humuli* (DC.) Burr.) varied according to its stage of development.

In 1918 by the selection of suitable material a method was adopted whereby any two solutions could be very strictly compared with regard to their fungicidal action. Thus it could be determined whether the nature of the polysulphide was of importance.

Methods.

While the general methods used in our experiments in 1915, which have been described in detail[2], were adopted in our work in 1916-18, it was found necessary to pay closer attention to the stage of development of the mildew-patch sprayed. On this point we wrote in 1915: 'The plant used for spraying was carefully selected as bearing on a number of its leaves young and vigorously-growing patches of the mildew in its conidial stage. In order to make the experiments as strictly comparable as possible only those patches of mildew were used where the

[1] J. Vargas Eyre and E. S. Salmon, "The Fungicidal Properties of Certain Spray fluids," *Journ. Agric. Science*, 7, 473–507 (1916).

[2] J. Vargas Eyre and E. S. Salmon, *loc. cit.*, p. 477.

growth was so vigorous that the abundant conidiophores had produced masses of ripe, free conidia. This stage is denoted by the term "powdery" in the details of the experiments given below.'

During the work in 1916 it became evident that the "powdery" patches on the older leaves of the plant were more easily killed than those on the younger leaves. In the experiments with the different solutions which are described in detail below, several instances will be found recorded where the powdery stage on a young leaf survived the application of a solution which killed the powdery stage on an older leaf,—sometimes even at the next node. In the later experiments in 1916, and in all those in 1917 and 1918, information is given in the Tables as to the relative age of the leaves on which the mildew occurred. In the experiments recorded below, just over 900 leaves, each usually bearing several "powdery" patches of mildew, have been sprayed and kept under close observation in the greenhouse.

Taking everything into consideration, the best standard to adopt for comparing the relative fungicidal powers of different solutions was found to be that based on the "death-point" of the young powdery conidial stage of the mildew found on the young, vigorous leaves of the hop-plants used in the experiments. It is by this standard that the fungicidal values of the solutions used have been fixed. In the experiments carried out in 1918 to compare the fungicidal action of two given solutions as closely as possible, the method was adopted of selecting mildew-patches at the same stage of development on opposite leaves at the same node,—the mildew-patches on leaves at the nodes above and below serving as controls.

Some Biological Factors determining the Fungicidal Action of the Solution.

In some of the experiments the behaviour of the solution towards the earliest stages of development of the mildew, i.e., those immediately following infection, has been noted. Very frequently, in the case of young and rapidly growing hop-leaves, the first sign of infection is the presence of a convex "blister" or "hump"; this is green at first, with no apparent sign of the mildew, then later (as observed under a pocket-lens) fine, branched hyphae appear straggling over its surface, which increase until the "hump" becomes covered over with the young, delicate vegetative mycelium. Conidiophores then quickly begin to appear, and after a few days a small, "powdery" patch results.

The early stages of development of the mildew antecedent to the

production of conidiophores have a much greater resistance to the fungicidal action of ammonium polysulphide solutions. The following tabulated results of three experiments in which three different solutions were used may be given here to illustrate this point.

Ref. No. of Experiment	Ammonium polysulphide solution, and its dilution	Mildew in the "powdery" conidial stage	Mildew in the early, non-powdery stages	Results
10/17	II; 1 : 50	(1) on 8 leaves, at 5th to 8th node from apex	(2) on 1 leaf at fifth node, among "powdery" patches	(1) all killed (2) unaffected
55	IV; 1 : 100	(1) on 6 leaves at 5th to 7th node	(2) on 1 leaf at 5th node	(1) all killed (2) checked
59	IV A; 1 : 100	(1) on 3 leaves at 5th node	(2) on 3 leaves at 4th node	(1) all killed (2) somewhat checked

In several of the experiments it was noticeable that a considerable variation occurred as regards the resistance shown to the solution even when the mildew-patches were all in the powdery conidial stage. The powdery patches on the young leaves resisted a concentration of the solution which was lethal to those on the older leaves of the same plant. Some of the more striking cases are tabulated below.

Ref. No. of Exper.	Solution	Position of leaves bearing the mildew-patches (in the powdery conidial stage) sprayed	Results
91	Ammonium polysulphide Solution II; 1 : 60	plant 1, 6th node (*a*) ,, 1, 5th ,, (*b*)	(*a*) some patches killed; others more or less checked (*b*) unaffected
79	A.p. S. III; 1 : 100	plant 1, 5th node (*a*) ,, 1, 4th ,, (*b*) ,, 2, 5th ,, (*c*) ,, 2, 4th ,, (*d*)	(*a*) killed (*b*) some patches killed, a few greatly checked, some slightly checked (*c*) killed (*d*) some patches killed, some greatly checked
65	A.p. S. IV A; 1 : 150	plant 1, 5th node (*a*) ,, 1, 4th ,, (*b*) ,, 2, 5th ,, (*c*) ,, 2, 4th ,, (*d*)	(*a*) (*c*) most patches killed, a very few just alive (*b*) (*d*) patches slightly checked or unaffected

The explanation of the fact that the powdery patches on the older leaves were easier to kill than those on the younger leaves may be that the former were all older, and that older patches are more vulnerable, or possibly that the vigour of the leaf directly affects the vigour and power of resistance of the powdery mildew.

It is clear from the cases mentioned above—and others will be found described below—that the mildew shows in its different stages of development very different powers of resistance to the same solution, being very hard to kill in the stages just following infection, and comparatively easy to kill when in its powdery conidial stage on the "older" leaves.

By the selection of only those patches of mildew in the same stage of development and on young, vigorously-growing leaves it is possible to keep a sufficiently fixed standard by which to measure satisfactorily the fungicidal value of different solutions. Where, however, two solutions have to be compared under as strictly similar biological conditions as possible, it is necessary to use mildew-patches in the same stage of development on leaves at the same node. It is much to be hoped that in future work dealing with the "powdery mildews" and their fungicides exact indication will be given both of the stage of development of the mildew-patches used and of the position of the leaves bearing the mildew.

Materials used.

Distilled water has been used in the preparation of the various solutions, and with the exception of a few instances which are referred to, soft soap was added to ensure the proper wetting of the mildew.

Soap. In nearly all cases where soap has been used alone or in conjunction with other substances, the kind known commercially as "Chiswick Soft Soap" has been employed. The sample was a moderately firm type of soft soap practically neutral in character. The total alkali was found to be equal to 12·8 % KOH (or 9·1 % NaOH).

In the other cases where soap was used it was that known commercially as "Cook's Soap," a rather fluid type of soft soap which exhibited a neutral or slightly acid character. The total alkali was found to be equal to 11·6 % KOH (or 8·36 % NaOH).

Saponin. The material used was the ordinary white powder sold commercially.

Ammonium hydrosulphide. This was prepared by saturating a 4 % solution of ammonia in water with sulphuretted hydrogen gas. The

amount of sulphide sulphur present in the solution was found to be 6·24 %.

Yellow ammonium sulphides. Eleven different solutions containing ammonium polysulphide were used during the course of this work. The analyses of these solutions are given on p. 306. Briefly described their mode of preparation was as follows[1].

Solution I. This solution was made by saturating 200 c.c. of a 10 % solution of ammonia in water at 17° C. with sulphuretted hydrogen gas and then adding 400 c.c. of 10 % ammonia solution and 1000 c.c. of water. To this mixture 24 grms. of flowers-of-sulphur were added and when this was completely dissolved the clear solution formed the Stock Solution No. I. The specific gravity of this stock solution was 1·001 at 15° C.

Solution II. This was prepared by saturating 1·5 litres of 30 % aqueous ammonia with hydrogen sulphide gas until the specific gravity of the liquid rose to 0·955. To this solution 3 litres of 30 % ammonia were added and 567 grms. of flowers-of-sulphur. This gave a dark brown fluid which had sp. gr. 0·950 and showed a tendency to deposit sulphur when largely diluted with water.

Solution III. This was prepared from Solution II by passing more sulphuretted hydrogen gas through the liquid until the specific gravity reached 1·05. This Stock Solution III was more stable when diluted with water than Solution No. II from which it was prepared.

Solution IV. To 500 c.c. of Stock Solution No. II, 55 grms. of flowers-of-sulphur were added and a current of sulphuretted hydrogen gas passed through the liquid until all the sulphur was dissolved. The resulting liquid had a sp. gr. 1·036 and was very dark in colour. When diluted largely with water a considerable deposition of sulphur occurred after about ten hours.

Solution IV A. This solution was prepared from Solution IV by allowing a current of sulphuretted hydrogen gas to pass through the solution until its specific gravity became 1·087. Solution No. IV A proved to be less liable to deposit sulphur when diluted with water and allowed to stand.

Solution V. A solution of ammonia in water (sp. gr. 0·987 at 15° C.) was saturated with sulphuretted hydrogen gas and an aqueous solution of ammonia was added until on testing with copper sulphate

[1] Several of the solutions used were made according to the details given by Bloxam. *Vide* "The Sulphides and Polysulphides of Ammonium," *Trans. Chem. Soc.*, **67**, 1895, pp. 277–309.

solution[1], it was found that (S) and (NH_4) were present in the necessary proportions to form $(NH_4)_2S$. In one litre of this solution, 12 grms. of flowers-of-sulphur were dissolved, giving a pale yellow liquid referred to as Stock Solution No. V.

Solution VI. To 300 c.c. of the solution of $(NH_4)_2S$ referred to in the preparation of Solution V, 30 grms. of flowers-of-sulphur were added and the liquid kept in a warm place for some time. After about 24 hours the clear, red liquid was decanted from the excess of sulphur, and it formed the stock solution. This stock solution did not allow of being diluted with water without depositing large quantities of sulphur, nor could it be kept for long unchanged.

Solution VII. A current of sulphuretted hydrogen gas was passed through a litre of aqueous ammonia (sp. gr. 0·987 at 15° C.) until the solution was practically saturated. The solution was then mixed with a litre of aqueous ammonia of the same sp. gr. as that used in the first instance. To this mixture 200 grms. of flowers-of-sulphur were added and a further quantity of hydrogen sulphide gas passed through the liquid until the clear liquid had sp gr. 1·034 at 15° C. This clear liquid formed the Stock Solution No. VII.

Solution VIII. To 400 c.c. of strong aqueous ammonia (sp. gr. 0·880) an equal volume of distilled water was added and whilst a current of sulphuretted hydrogen gas was passing through the liquid, flowers-of-sulphur were added in excess from time to time. After several hours, when no more sulphur would dissolve and the temperature of the liquid decreased, the deep red clear liquor which resulted was decanted from the undissolved sulphur into a stoppered vessel. After standing for several days, this decanted liquid deposited crystals which on analysis were found to contain 18·33 % NH_4 and 81·2 % S, therefore agreeing with the composition $(NH_4)_2S_5$. To a weighed quantity (8·2354 grms.) of these crystals in a stoppered bottle, cold recently boiled water was added, resulting in the deposition of sulphur and the formation of a golden yellow solution. The water was run in until the addition of a small quantity produced no further precipitation of sulphur, the volume of water necessary in this case being 200 c.c. After remaining for two days in the closed vessel, the golden yellow solution was separated from the deposited sulphur and this formed the Stock Solution No. VIII.

Solution IX. This solution was prepared in the same way as Solution VIII, i.e. by decomposing crystals of $(NH_4)_2S_5$ by adding water

[1] The method used was that given by Bloxam. *Vide Trans. Chem. Soc.*, 1895, p. 289.

to them and taking the golden yellow solution formed. The results of analysing Solutions VIII and IX showed them to consist practically entirely of ammonium trisulphide $(NH_4)_2S_3$ in solution.

Solution X. A current of sulphuretted hydrogen gas was passed through a litre of a solution of ammonia in water (sp. gr. 0·980 at 15° C.) until it was practically saturated. An aqueous ammonia solution of the same strength was then added until by testing with copper sulphate solution it was found that (NH_4) and (S) were in the proportions required to form $(NH_4)_2S$. An excess of flowers-of-sulphur was then added and the liquid was warmed by placing the flask containing it in a water bath, the temperature of which was not allowed to rise above 80° C. When no more sulphur seemed to dissolve, the deep red liquid which resulted was poured off from the undissolved sulphur and put in a well stoppered bottle. This liquid formed the Stock Solution No. X. When kept in a stoppered bottle, this solution remained clear but on dilution with water it became cloudy in a few minutes owing to the deposition of sulphur.

Solution XI. During the preparation of Solution VIII, crystals of $(NH_4)_2S_5$ were formed and these were separated from the mother-liquor. Solution XI was made from that mother-liquor by mixing 250 c.c. with 250 c.c. of an aqueous ammonia solution (sp. gr. = 0·926 at 11° C.). The specific gravity of Solution XI at 15° C. was 1·075.

Lime sulphur. In the course of these experiments, the following two solutions of lime sulphur were used.

Solution XII. A mixture of 55 grms. of calcium oxide, 110 grms. of flowers-of-sulphur, and 400 c.c. of distilled water was boiled under a reflux condenser for 1¼ hours. This was left to stand for about 12 hours when the clear dark red liquid was decanted from the insoluble residue into a well stoppered bottle. After standing for two weeks, a few small crystals were observed to have separated. The clear liquid was separated from these and formed the stock solution for the spray-fluids. The specific gravity of this stock solution was 1·221 at 10° C.

Solution XIII. This was a commercial sample of lime sulphur.

The results of the analysis of both the above solutions are given on p. 306.

In preparing the actual spray-fluids from the stock solutions, distilled water was invariably used. The stock solution was diluted to such a volume that the concentration was double that finally required in the actual spray-fluid and this solution was mixed with an equal volume of a 2 % solution of soft soap. For instance, where a wash containing

2 % of Solution No. IX and 1 % soft soap was used, this was prepared by diluting 8 c.c. of stock solution to 200 c.c. with distilled water and then adding 200 c.c. of a 2 % solution of soft soap in distilled water.

Details of Biological Observations.

Ammonium polysulphide Solution I.

This stock solution diluted 1 : 50 and upwards is not fungicidal, although at 1 : 50 it is nearly fungicidal, killing some patches and severely checking the others.

As with our experiments in 1915, it was found that the solution diluted 1 : 20 and containing 0·5 % or 1 % soft soap, is fungicidal for the "powdery" conidial stage. At this strength it did not kill the young non-powdery stage of the mildew in two experiments, but in a third experiment, where the host plant was *H. Lupulus* var. *cordifolius*, the effect was fungicidal and very striking.

The solution, diluted 1 : 20, was found to lose much of its potency if allowed to stand, after dilution, for four days, and had no fungicidal value after six days.

Ammonium polysulphide Solution II (with 1 % soap).

Although in two experiments this solution diluted 1 : 160 killed the patches of mildew, there is reason to believe that this happens only when the patches are on old leaves and therefore with a comparatively slight power of resistance. In three later experiments (with 14 leaves), where a record exists as to the comparative age of the sprayed leaves, it was found that the solution at this strength has no fungicidal power over the powdery conidial stage on the younger leaves. The solution at 1 : 100 has considerable fungicidal power, but in no case did it kill all the patches on all the leaves sprayed in the experiment. At 1 : 50 it was fungicidal for the powdery conidial stage in two out of four experiments. At 1 : 30 it was fungicidal in two experiments (with 12 leaves). At this strength the solution may injure the leaves.

In some of the experiments it became obvious that the death-point of the "powdery" mildew-patch varied according to the age of the leaf on which it resided. Thus in Experiment **31**, with the solution at 1 : 160, two leaves were sprayed on one plant; the "upper" leaf bore eight powdery patches, and the "lower" many powdery patches. On the fifth day after spraying, the patches on the lower leaf were "much checked or even semi-obliterated," while on the upper leaf all the

patches had begun to produce fresh conidiophores. By the ninth day it was evident that some of the patches on the lower leaf were dead, and from the remaining patches only a few weak conidiophores had been produced; on the upper leaf all the eight patches had densely clustered conidiophores and were again powdery.

Again, in Experiment 91, with the solution at the same strength, the powdery patches on the two leaves of one plant, at the sixth and fifth nodes from the apex, showed different powers of resistance. On the fifth day after spraying, the patches on the lower leaf were sterile or partly obliterated, while the control leaf was densely powdery; the patches on the upper leaf had regrown conidiophores and were now almost as powdery as those of the "control" leaf. By the tenth day some of the patches on the lower leaf were dead, but a few patches had revived and produced conidiophores and were now sub-powdery; on the upper leaf all the original patches were now densely powdery like those of the "control" leaf.

Ammonium polysulphide Solution III (with 1 % soap).

This solution diluted 1:100 has considerable fungicidal powers. At 1 : 50 it was completely fungicidal in one experiment, and very nearly fungicidal in two experiments. It was fungicidal at 1 : 30 in the one experiment at this strength, and caused slight but distinct injury to six of the eight leaves sprayed.

In Experiment 79, with the solution diluted 1 : 100, there was clear evidence showing that the reaction of the mildew to the solution varied according to the different stages of growth reached by the fungus. On one of the plants used three leaves were sprayed; leaf (*a*) at the fifth node from the apex bore numerous powdery patches, leaf (*b*) at the fourth node also bore numerous powdery patches, while leaf (*c*) at the third node bore very young mycelial patches without conidiophores. On the eleventh day after spraying, the patches on leaf (*a*) were dead, while the control leaf bore powdery patches; on leaf (*b*) a few patches were dead, some patches had newly produced a few scattered conidiophores either at the centre of the patch or at its periphery, but most patches had produced clustered conidiophores again over most of their surface and were again powdery or sub-powdery—the mildew-patches on the control being only slightly more vigorous; on leaf *c* there were numerous very powdery patches,—the solution not having stopped the development of the young stage, there was no difference between the sprayed leaf and the control leaf.

Ammonium polysulphide Solution IV (Table 1).

This solution diluted 1 : 300 has considerable fungicidal power, which varies according to the age of the leaf on which the "powdery" conidial patch resides (see Table 1). At 1 : 100 it is fungicidal for all powdery patches on both old and young leaves, and it checks the development of the young non-powdery stages. At 1 : 75 the check to the young stages is more marked, and at 1 : 50 the mildew in its very earliest stages of development is almost or quite killed. Some details of three experiments in which the solution was used at 1 : 100, 1 : 75 and 1 : 50 may be noted here, as they give clear evidence that the mildew in different stages of development has different powers of resistance to the solution.

In Experiment 55 (1 : 100) one of the leaves sprayed (*c*) had 20 "humps," showing the early stages of development of the mildew. On the eighth day after spraying three tiny tufts of clustered conidiophores occurred arising from three "humps," while on 17 of the "humps," where the mildew had existed in its earliest mycelial growth, the fungus was killed. On the tenth day, the sprayed leaf bore three small, more or less powdery patches, while the control leaf bore 20 powdery patches. Where, on the six other sprayed leaves, the mildew had been in the form of vigorous powdery patches, it was killed.

In Experiment 73 (1 : 75) seven leaves were sprayed. On four leaves (*a*, *c*, *d*, *f*) the mildew was in the powdery conidial stage; on one leaf (*b*) the only sign of the presence of the mildew was the occurrence of numerous "humps," indicating where infection had taken place, although no mycelium was visible (under a lens) on the surface of the "hump"; on one leaf (*e*) the patches were at that stage of development when mycelium has been produced but as yet no conidiophores; on one leaf (*g*) there were a few powdery patches, one very young sterile patch and several "humps" bearing a few sterile mycelial hyphae radiating from their centres. By the fifth day after spraying the patches on the four leaves (*a*, *c*, *d*, *f*) were all sterile and either dead or dying—some being semi-obliterated; while the patches on the control leaves were as powdery as before; on leaf (*b*) only two of the numerous "humps" bore a few weak conidiophores, while the control leaf bore very numerous powdery patches. It was clear that the solution had dealt summarily for the most part with the young stages of the mildew. On the seventh day the sprayed leaf bore four tiny groups of clustered conidiophores arising from four "humps," while the control leaf was almost continuously

TABLE 1. *Ammonium polysulphide Solution IV and 1 % soap.*

Ref. no. of Exper.	Dilution	No. of of leaves sprayed	Position of leaf	Stage of mildew	Effect of solution on "powdery" stage	Effect of solution on young stage
22	1 : 300	6	"Lower" and "upper" leaves*	powdery patch	Killed on "lower" leaves; more or less checked on "upper" leaves	—
23	1 : 300	6	"Lower," "middle" and "upper" leaves*	,,	Severely checked on "lower" leaves; slightly checked or unaffected on "middle" and "upper" leaves	—
24	1 : 200	7	—	,,	Some patches killed; some severely checked; a few slightly checked	—
55	1 : 100	7	Plant 1 (*a*) 7th node	,,	Killed on (*a*), (*b*), (*d*), (*e*), (*f*), (*g*)	Checked on (*c*)
			,, 1 (*b*) 6th ,,	,,		
			,, 1 (*c*) 5th ,,	young stage ("humps")		
			,, 2 (*d*) 6th ,,	powdery patch		
			,, 2 (*e*) 5th ,,	,,		
			,, 3 (*f*) 7th ,,	,,		
			,, 3 (*g*) 6th ,,	,,		
49/17	1 : 100	10	At 3rd to 7th nodes on three plants	,,	Killed on all leaves	—
73	1 : 75	7	Plant 1 (*a*) 5th node	,,	Killed on (*a*), (*c*), (*d*), (*e*), (*f*)	Greatly checked on (*b*), (*g*)
			,, 1 (*b*) 4th ,,	young stage (green "humps")		
			,, 2 (*c*) 5th ,,	powdery patch		
			,, 2 (*d*) 4th ,,	,,		
			,, 2 (*e*) 3rd ,,	,,		
			,, 3 (*f*) 4th ,,	,,		
			,, 3 (*g*) 3rd ,,	young stage ("humps")		
72	1 : 50	7	Plant 1 (*a*) 5th node	powdery patch	Killed on (*a*), (*d*), (*f*), (*g*)	Killed on (*b*), (*e*) Very nearly killed on (*c*)
			,, 1 (*b*) 4th ,,	young stage ("humps")		
			,, 1 (*c*) 3rd ,,	,, ,, ,,		
			,, 2 (*d*) 4th ,,	powdery patch		
			,, 2 (*e*) 3rd ,,	young stage ("humps")		
			,, 3 (*f*) 4th ,,	powdery patch		
			,, 3 (*g*) 3rd ,,	,,		

* Indicates the relative position at successive nodes.

covered over with densely powdery patches; on the tenth day there were seven very small powdery patches. On leaf (*e*) there was no sign of any living mildew on the fifth day, while the control leaf bore very numerous powdery patches. On leaf (*g*) on the fifth day the only sign of life was one small group of clustered conidiophores from one "hump," the mildew in the other stages of development having been killed; the control leaf was almost continuously covered with powdery patches. By the tenth day four tiny tufts of conidiophores were observed coming from the "humps."

In Experiment 72 (1 : 50), on leaf (*b*) the mildew had reached the stages of development characterised either by the presence of a minute sterile patch of mycelium or by the presence only of scarcely observable young mycelial hyphae radiating over the surface of the "hump"; on leaf (*c*) there was no visible sign of infection beyond the presence of green "humps"; on leaf (*e*) the mildew occurred either in the form of tiny sterile mycelial patches or as "humps," while on leaf (*g*) only the former stage was present. The solution proved fungicidal for all stages of the mildew except the very earliest ones on leaf (*c*). On this leaf by the fifth day there were a very few weak conidiophores from one infection spot, while the control leaf bore numerous small powdery patches. On the seventh day conidiophores to the number of 12 existed on the sprayed leaf, and no further development had taken place by the tenth day.

Ammonium polysulphide Solution IV A (with 1 % soap).

This solution diluted 1 : 150 exerted strong fungicidal powers. At 1 : 100 it was fungicidal for all "powdery" patches in one experiment, and very nearly so in the second experiment. At 1 : 75 all "powdery" patches were promptly killed, in many cases being almost completely obliterated by the fifth day after spraying. At 1 : 50 the solution proved, in two experiments, fungicidal for even the youngest stages of development.

Comparison of Ammonium polysulphide Solutions VIII and XI (Table 2).

These solutions tested[1] at four strengths gave practically identical results.

With 0·010 % polysulphide sulphur the solutions were entirely

[1] In these experiments, as in all the following ones in which two solutions were compared with each other, the leaves sprayed with the two solutions were always at the same node.

TABLE 2. *Comparison of Ammonium polysulphide Solutions VIII and XI (both solutions with 1 % soap).*

Ref. No. of Exper. 1918	Solution	% of polysulphide sulphur	No. of leaves sprayed	Position of leaf*	Effect of solution on mildew
15	VIII	·010	12	3^1. 4^1. 5^3, 6^3, 7^3, 8^1	VIII, XI: unaffected
14	XI	·010			
13	VIII	·032	12	4^3, 5^4. 6^2, 7^2. 8^1	VIII, XI: unaffected on two leaves at the 4th and 5th nodes; on other leaves some patches killed, rest more or less checked
12	XI	·032			
10	VIII	·053	11	4^1. 5^2. 6^2, 7^3, 8^3	VIII nearly all the patches killed; a very few patches with a few, usually scattered, conidiophores
11	XI	·052			XI nearly all the patches killed; a few patches with clustered conidiophores at the edges (on five leaves action apparently slightly less fungicidal than VIII)
9	VIII	·077	10	4^1. 5^1. 6^3. 7^3, 8^2	VIII, XI: Fungicidal (all the patches killed)
8	XI	·078			

* The numeral in larger type indicates the node (counting from the apex). and the numeral in smaller type the number of leaves sprayed at that node.

without fungicidal effect; on the fifth day after spraying all the patches were as powdery as before.

With 0·032 % polysulphide sulphur the fungicidal action was quite evident, and the identity of action of the two solutions was well seen. On the two leaves, each at the fourth and fifth nodes, the two solutions did not prevent most of the powdery patches from reforming clustered conidiophores by the fifth day; with regard to the other leaves there were a few dead patches on each leaf, and clustered conidiophores regrowing from the edge of most patches—the amount of growth of the mildew being the same for the two solutions.

With 0·053 % polysulphide sulphur Solution VIII proved very slightly more potent than Solution XI with 0·052 % polysulphide sulphur; the difference, however, was so slight (considering the variable biological factors concerned) that it cannot be held to impugn the identity in fungicidal action of the two solutions.

With 0·077 % and 0·078 % polysulphide sulphur the action was fungicidal; from the third day onward the sprayed patches remained white and persistently sterile until they died.

Comparison of Ammonium polysulphide Solutions XI and IX
(both solutions with 1 % soap).

There was strong similarity in the action of these two solutions. Solution XI with 0·031 % polysulphide sulphur was apparently slightly stronger in fungicidal action than Solution IX with 0·029 %. With 0·039 % and 0·036 % respectively, Solutions XI and IX proved to be identical in action. In Experiments 3 and 4, Solution XI, with 0·052 % was very slightly stronger in fungicidal action than Solution IX with 0·048 %. With 0·078 % and 0·072 % respectively, Solutions XI and IX both proved fungicidal.

Comparison of Ammonium polysulphide Solutions V and VII
(both solutions with 1 % soap).

At the only strength, viz., containing 0·077 % polysulphide sulphur, at which these solutions were compared, they proved identical in fungicidal action, as the following details show.

	Sol. V No. of leaf	Sol. VII No. of leaf*
Fungicidal	4, 5, 6, 7, 8, 10, 11, 12	4, 5, 6, 7, 10, 11, 12
Very nearly fungicidal ...	1, 2	1, 2
Not quite fungicidal	9	9

* leaf 8 died.

Comparison of Ammonium polysulphide Solutions VII and XI
(both solutions with 1 % soap).

The solutions proved similar in action at the only strength at which they were compared. Solution VII was used with 0·077 % polysulphide sulphur and Solution XI with 0·078 %. Ten leaves were sprayed. With Solution VII all the patches were killed on two leaves; many patches were killed on the remaining eight leaves, but some patches on each leaf regrew scattered or clustered conidiophores from their edges. With Solution XI many patches were killed on all the ten leaves, but it was not fungicidal for any leaf. The effect of the two solutions on the leaves at the same nodes are tabulated below:

	Sol. VII No. of leaf	Sol. XI No. of leaf
Fungicidal	1, 2	—
Very nearly fungicidal ...	5, 6, 8, 9, 10	1, 5, 6, 8, 10
Not quite fungicidal ...	3, 4, 7	2, 3, 4, 7, 9

Comparison of Ammonium polysulphide Solutions VIII and XI
(both solutions with 1 % saponin).

There was similarity in the action of these two solutions. In Experiments 28, 29, Solution VIII contained 0·077 % polysulphide sulphur and Solution XI 0·078 %. If we classify the results under the three headings "fungicidal," "not quite fungicidal," and "non-fungicidal," we have the table:

	Sol. XI No. of leaf	Sol. VIII No. of leaf
Fungicidal	2, 4, 5	2, 4, 7
Not quite fungicidal ...	3, 7, 8	1, 3, 5, 8
Non-fungicidal	1, 6	6

In Experiments 35, 36[1], where both solutions contained 0·157 % polysulphide sulphur, if we tabulate under four headings, we have

	Sol. XI No. of leaf	Sol. VIII No. of leaf
Fungicidal	1, 7, 9, 10, 12	4, 7, 9, 10, 12, 13
Very nearly fungicidal ...	3, 4, 5, 13	3
Not quite fungicidal ...	2, 6, 8	1, 2, 5, 6
Non-fungicidal	11	8, 11

The absence of complete fungicidal action with 0·157 % polysulphide sulphur is probably to be attributed to the inferior wetting powers of saponin as compared with soap.

[1] Sufficient force was used in this experiment practically to drench the leaves.

Ammonium hydrosulphide (containing 3·12 % *sulphide sulphur) compared with Ammonium polysulphide Solution XI (diluted to contain* 0·038 % *sulphide sulphur and* 0·113 % *polysulphide sulphur)* (both solutions with 1 % soap).

With ammonium hydrosulphide there was at first a severe checking action, so that on the fifth day after spraying seven of the leaves were still sterile, and on the three remaining leaves only a very few conidiophores had been produced at the edges of the patches. By the ninth day it was clear that that ammonium hydrosulphide was non-fungicidal for at least some leaves; while Solution XI was everywhere fungicidal[1]. The material on the whole was rather poor, which makes the non-fungicidal action of the ammonium hydrosulphide the more remarkable. On the 14th day the leaves were classified as follows:

	Ammonium hydrosulphide No. of leaf	Solution XI No. of leaf
Fungicidal	—	1 10
Non-fungicidal (several "powdery" patches)	2, 3, 7, 8, 9, 11, 14	—
Checked; clustered conidiophores from several patches	1, 6, 10	—
Severely checked: a very few conidiophores from a few patches (poor material) ...	4, 5	
Leaves discarded (injury)	12, 13	

Solution XI (ammonium polysulphide) compared with Solution XII (lime-sulphur)--both with 1 % saponin (Table 3).

These solutions behaved very similarly.

With 0·078 % polysulphide sulphur the two mixtures appeared to be identical in action. This was shown by the fact that the four leaves on which the patches were all killed by each solution were at the same nodes on the same plants, as was also the case with the six leaves on which many patches produced more or less clustered conidiophores round their edges.

With 0·113 % polysulphide sulphur the action of the two solutions, although very similar, was slightly more fungicidal in the case of the ammonium sulphide, as the following details show. On leaf 1 the action of the two solutions was identical; on leaves 2, 3, 4, 5 the ammonium sulphide solution proved almost fungicidal (i.e., most of the

[1] Four leaves, Nos. 11–14, opposite to those sprayed with the ammonium hydrosulphide, were left unsprayed as "controls." On the fifth day only on one leaf, No. 14, were the patches powdery, on the remaining three leaves the mildew appeared to be dying away.

TABLE 3. *Solution XI (Ammonium polysulphide) compared with Solution XII (Lime-sulphur) (both solutions with 1 % saponin).*

Ref. No. of Exper.	Solution	% of polysulphide sulphur	No. of leaves sprayed	Position of leaf	Effect of solution on mildew
20 21	XI XII	·078 ·078	10	$4^2, 5^4, 6^3, 7^1$	XI, XII: all patches killed on four leaves; on six leaves some patches killed, but many only more or less checked
24 25	XI XII	·113 ·113	10	$4^1, 5^2, 6^2, 7^3, 8^2$	XI almost fungicidal on four leaves; patches severely checked on four leaves, slightly checked on two leaves XII patches severely checked on five leaves, slightly checked on five leaves

TABLE 4. *Ammonium polysulphide Solution XI compared with this solution after precipitation of sulphur (both solutions with 1 % soap).*

Ref. No. of Exper.	Solution	No. of leaves sprayed	Position of leaf	Effect of solution on mildew
39 40	XI Precipitated sulphur	13	$3^2, 4^4, 5^3, 6^3, ?^1$	XI Fungicidal (no injury) Precipitated sulphur. Fungicidal, with occasional injury
42	Ammonium chloride	14	$3^5, 4^5, 5^1, ?^3$	*Ammon. chloride.* On two leaves patches ultimately unaffected; on four leaves patches slightly checked; on three leaves patches greatly checked, and nearly killed (remaining leaves unsatisfactory). Occasional injury
44	Precipitated sulphur	7	$3^2, 4^2, 5^1, ?^2$ (opposite leaves 8–14 of Experiment 42)	*Precipitated sulphur.* Fungicidal; with occasional injury
43	Soap (1 %)	7	$3^3, 4^3, ?^1$ (opposite leaves 1–7 of Experiment 42)	*Soap.* On four leaves patches unaffected; on two leaves patches checked (one leaf unsatisfactory)

patches were killed, but a few on each leaf produced a very few conidiophores at the edges), while on these leaves sprayed with the lime-sulphur solution although some of the patches were killed, most regrew some clustered conidiophores. On leaves 7, 8, 9, the ammonium sulphide solution had the same effect as that described above for the leaves 2 5 sprayed with lime-sulphur, while the lime-sulphur solution on leaves 7, 8, 9 was decidedly less effective, most of the patches regrowing densely clustered conidiophores. On leaves 6 and 10 both solutions had this same almost non-fungicidal effect.

As has been mentioned, at neither strength were the solutions completely fungicidal. Since the action was less marked at the higher concentration of the solution, it seems clear that some new factor was operative, perhaps that of a reduced spreading power.

Comparison of Lime-sulphur Solutions XII and XIII
(both with 1 %-saponin).

In Experiment 41 Solution XII, with 0·17 % polysulphide sulphur, was fungicidal for nine out of the eleven leaves. The two leaves on which the mildew lived and produced powdery patches of conidiophores were mildewed over practically their whole surface at the time of spraying. It is possible that under such conditions the inferior spreading powers of saponin did not ensure contact everywhere with the solution. In Experiment 45 the solution, at the same concentration, was fungicidal for seven leaves; on three leaves a few of the patches regrew a few scattered conidiophores, and on one leaf (at the third node) some patches regrew clustered conidiophores.

With 0·23 % polysulphide sulphur both solutions proved completely fungicidal. Used at this strength with 1 % saponin both these lime-sulphur solutions leave no visible deposit on the sprayed parts, – a point of considerable economic importance when such fruits as gooseberries are sprayed.

At the present time lime-sulphur solutions are invariably used in this country as a "summer spray" (without the addition of saponin or any other spreading agent) at a dilution of 1 in 30, the concentrated solution having the specific gravity of 1·30. With such a commercial brand as that used in the above experiments, such a dilution gives 0·77 % of polysulphide sulphur.

If the result obtained in the above experiments (where a concentration of 0·23 % polysulphide sulphur proved fungicidal) holds good when mildews on plants growing in the open are sprayed, it is obvious that

such commercial brands of lime-sulphur can safely be used at a much greater dilution than at present, in which case no disfigurement of the sprayed parts due to the dried deposit (such as is found at stronger concentrations) would occur.

Comparison of Ammonium polysulphide Solutions X and XI (both solutions with 1 % soap).

Each solution was diluted to contain 0·078 % of polysulphide sulphur.

In Experiments 18, 19, the two solutions behaved identically on the leaves at the same nodes on the same plants, killing all the patches on six leaves, and very nearly killing all the patches on the other six leaves, the patches remaining alive producing only a few scattered or clustered conidiophores at their edges.

In Experiments 26, 27, practically the same results were obtained, the mildew on each pair of leaves at the same mode being affected in precisely the same manner.

It is obvious from these experiments (with 22 leaves, each bearing numerous "powdery" patches) that both these solutions, with 0·078 % polysulphide sulphur, were very nearly at fungicidal strength.

Ammonium polysulphide Solution XI compared with this solution after precipitation of sulphur (both solutions with 1% soap) (Table 4).

In the first experiment (39, 40) with 13 leaves Solution XI, before and after precipitation of sulphur, proved fungicidal; in the latter case severe scorching injury resulted to the edges or tips of three leaves.

In the second experiment 14 leaves were sprayed with 0·28 % ammonium chloride (which was the amount present in Solution XI after precipitation of its sulphur). Of the leaves opposite to these 14 leaves, seven (Nos. 1–7) were sprayed with 1 % Chiswick soap, in order to see whether this substance had any fungicidal effect[1] under the conditions obtaining at the time of spraying; the remaining seven leaves (Nos. 8–14) were sprayed with Solution XI after precipitation of its sulphur. Owing to the material being poor, several leaves had ultimately to be discarded.

[1] In an experiment on May 18, 1918, three leaves (at the 7, 8 and 9 nodes) bearing powdery patches were sprayed with 1 % Chiswick soft soap. On the next day only a few of the patches showed less than normal vigour, and by the third day all the patches were as powdery as before spraying.

If we compare first the seven leaves sprayed with ammonium chloride with the seven leaves sprayed with the soap solution, we find that the condition of the mildew on the eleventh day was as follows:

	Soap No. of leaf	Ammon. chloride No. of leaf
Patches unaffected	3, 4, 5, 6	1*, 4*, 6*
Checked, but some patches with clustered conidiophores	1, 2	2, 3, 5*
Leaf discarded (mildew apparently dying away naturally)	7	7

* Tip of leaf showing "scorching" injury.

The seven leaves sprayed with ammonium chloride compared with the opposite leaves at the same node sprayed with Solution XI after precipitation of sulphur (and containing 0·28 % ammonium chloride) were classified on the eleventh day as follows:

	Sol. XI after precipitation of sulphur No. of leaf	Ammonium chloride No. of leaf
Fungicidal	8*, 10*, 11*, 12	—
Discarded as too poor material	9*, 13, 14	9*, 13, 14
Almost fungicidal (a very few scattered conidiophores from a few patches)	—	8*, 10*, 12
A few patches sterile: most patches with fresh clustered conidiophores	—	11*

* Tip of leaf showing "scorching" injury

There is, therefore, some evidence that the precipitated sulphur exerted a fungicidal action, although owing to the poor material used, this evidence cannot be considered as conclusive.

THEORETICAL CONSIDERATIONS.

In our previous communication we brought forward evidence showing that the fungicidal value of alkaline sulphide solutions is not to be ascribed to the total amount of sulphur such solutions contain, nor to the proportion of sulphur which is present in the form known as sulphide sulphur. We were led to conclude that the soluble polysulphides present were mainly responsible for their fungicidal behaviour.

During the seasons 1916–17 further evidence was gained in support of this view. On reference to the analyses of the solutions used in the course of this work which are given on page 306 it will be seen that Solution III contained approximately ten times the amount of sulphide sulphur present in Solution II, and eleven times that contained in Solution I. Also Solution III has three times the amount of total sulphur

present in Solution II, and eight times that contained in Solution I. By contrasting the behaviour of these it has been found that they all have apparently the same fungicidal value. Again, Solution IV contains less than half the sulphide sulphur contained in Solution III, and less total sulphur, yet Solution IV behaves more powerfully as a fungicide. The fact that this solution contains three times as much polysulphide sulphur as is present in Solution III supports our earlier conclusion that the proportion of polysulphide sulphur present is the important factor determining the value of a sulphide spray fluid.

Further experiments have been made with colourless ammonium hydrosulphide and these again point to the inefficacy of the sulphide form of sulphur. For example, a colourless ammonium hydrosulphide solution, when diluted so as to contain 3·12 % of sulphide sulphur, was sprayed upon mildewed leaves and, on similarly mildewed leaves at the same nodes, a diluted yellow ammonium sulphide solution was sprayed which contained only 0·038 % sulphide sulphur. The latter solution, however, proved to be fungicidal, whereas the colourless ammonium hydrosulphide solution, containing rather more than eighty times more sulphide sulphur, although severely checking the fungus for a time, was not fungicidal.

As experience was gained it became possible to make very close comparisons of the behaviour of these spray fluids and in 1918 attempts were made to determine whether there exists a relationship between the percentage of polysulphide sulphur present and the fungicidal action of such solutions. For this purpose solutions as widely different as possible were needed, and on reference to Table 5, it will be seen that from this point of view a comparison of Solution VIII with Solution XI was desirable. These solutions were therefore diluted so that each contained the same amount of polysulphide sulphur in solution and both these diluted fluids were sprayed upon mildewed leaves at the same nodes. These solutions were used in this way at four different dilutions, and in every case the fungicidal action was closely similar or identical (see Table 2). In precisely the same way Solutions IX and XI were used and in each case these two solutions behaved similarly when compared on a polysulphide basis. Two other solutions, Nos. V and VII, were diluted until each contained 0·077 % polysulphide sulphur and the fungicidal actions of the diluted liquids were observed to be similar. Solution XI was then diluted so as to contain 0·078 % polysulphide sulphur and then compared at the same node with a diluted solution of No. VII containing 0·077 % polysulphide sulphur.

The two solutions, diluted in this way, exhibited very similar fungicidal behaviour.

In all the experiments just described the spray fluid contained 1 % of soft soap, added to ensure uniform spreading. In view of the possibility that this addition of soap exercised some disturbing influence, saponin was substituted for soap and a fresh comparison made between Solutions VIII and XI. On reference to p. 297 it will be seen that under these circumstances the fungicidal action of these two fluids was similar when they contained equal amounts of polysulphide sulphur.

These results seem to indicate a parallelism between the fungicidal action and the polysulphide sulphur content of these solutions. It seems that ammonium polysulphide solutions containing equal amounts of polysulphide sulphur behave in exactly the same way fungicidally.

From the foregoing results it was thought possible that ammonium polysulphide solutions and lime-sulphur solutions, having the same polysulphide content, would have the same fungicidal action. To ascertain whether this were the case, a lime-sulphur solution, made in the laboratory, and containing 17·02 % polysulphide sulphur, was diluted until the polysulphide sulphur content was 0·078 % when it was sprayed on mildewed leaves; the opposite and similarly mildewed leaves at the same nodes being sprayed with an ammonium polysulphide solution (XI), also containing 0·078 % of polysulphide sulphur. The results given in Table 3 show how very similar these two solutions were in their fungicidal action. In both these solutions saponin was used instead of soap so as to secure a fair comparison. These two solutions were again used at a different strength, each having a polysulphide sulphur content of 0·113 %, and again similar action was observed.

When the lime-sulphur solution previously referred to as having been made in the laboratory was diluted so that it contained 0·23 % of polysulphide sulphur it was found to be completely fungicidal. A commercial sample containing 23·21 % of polysulphide sulphur was likewise diluted until it also contained 0·23 % polysulphide sulphur, and this also was found to be completely fungicidal. Thus when working with lime-sulphur solutions as with ammonium sulphide solutions there seems to be this dependence upon polysulphides for fungicidal character. With the object of gaining information as to whether the higher polysulphides are more potent as fungicides than the lower polysulphides, special efforts were directed to prepare stock solutions of different chemical composition. For example, it was desired to make use of a solution for spraying purposes containing mostly low ammonium

polysulphides for comparison with one containing some of the higher ones. The analyses of the solutions prepared show that solutions Nos. VIII and IX were different from Solution XI with which they have been compared. In the preparation of these two solutions, that is VIII and IX, the attempt seems to have been successful in producing a solution of the lower polysulphides of ammonia; the analysis showing them to contain practically nothing else than the ammonium tri-sulphide $(NH_4)_2S_3$. On the other hand, Solution XI appears from the analysis to contain some high polysulphides. It is interesting, therefore, to find that solutions VIII and XI agree so completely when compared on a polysulphide basis, and this seems to indicate that equal amounts of polysulphide sulphur, whether the polysulphides present be high or low polysulphides, behave in precisely the same way fungicidally. In a previous attempt to discover whether the polysulphides are active as such in killing the fungus, or whether this property is due to the sulphur deposited when these compounds decompose, evidence was found favouring the former view. This was based upon some attempts to weigh directly the amount of sulphur deposited from solutions of colourless ammonium hydrosulphide and yellow ammonium sulphide. As was suggested then it was necessary to repeat and extend these experiments. Work along these lines has given very conflicting results and we are not inclined now to place as much reliance as formerly upon the evidence then at hand; unfortunately we have not been able to obtain concordant results. In this connection, however, the behaviour of some of the stock solutions, for example No. X, proves of interest. This solution was not stable when diluted with water and much sulphur was deposited. The fungicidal action of this solution, rendered opaque by the addition of water, was compared with that of Solution XI when both were diluted to contain 0·078 % polysulphide sulphur and 1 % soft soap. The fluid made from Solution XI was quite clear at the time of spraying while that from Solution X was yellow and opaque owing to the deposition of sulphur. Both liquids were found to have the same effect on the fungus. This comparison was repeated this time after the diluted Solution X had stood for two hours to secure as completely as possible the precipitation of sulphur. After agitation liquid No. X was contrasted with liquid No. XI and again the two solutions behaved similarly. This result seems to show that a high polysulphide solution may deposit some of its sulphur without losing any of its fungicidal power and suggests that the freshly precipitated sulphur may have some action on the fungus. In view of this result and a possible action

TABLE 5. *Analysis of solutions used.*

Solution	Grams per 100 c.c. solution: Total sulphur	Sulphide sulphur	Polysulphide sulphur	Total NH_4	Total CaO	Excess of NH_4 over that required to form $(NH_4)_2S$	Sulphur in NH_4SH	Approximate fungicidal strength
I	3·7	2·2	1·5	—	—	—	—	F. at 1 : 20. At 1 : 50 nearly f. (one exper.)
II	9·1	2·5	6·6	26·1	—	—	—	F. at 1 : 30. At 1 : 50 f. in two expers.; nearly f. in two expers.
III	29·1	24·6	4·5	19·4	—	—	14·06	F. at 1 : 30. At 1 : 50 f. in one exper.; v. nearly f. in two expers.
IV	24·2	10·6	13·6	20·3	—	8·62	—	F. at 1 : 100
V	3·8	2·6	1·23	3·13	—	0·10	—	At 1 : 16 nearly f. (like Sol. VII at 1 : 44)
VI	9·4	2·4	6·9	3·10	—	Nil	—	F. at 1 : 50 (not tried at other strengths)
VII	7·2	3·8	3·4	3·14	—	—	3·03	At 1 : 44 nearly f. (like Sol. V at 1 : 16)
VIII	1·72	0·56	1·16	0·60	—	—	—	F. at 1 : 15
IX	2·12	0·67	1·45	0·69	—	—	—	F. at 1 : 20
X	13·85	3·75	10·1	4·54	—	0·29	—	At 1 : 130 nearly f. (two expers.)
XI	20·9	5·23	15·7	13·13	—	7·0	—	F. at 1 : 200 in two out of five expers.*; f. at 1 : 139 in four expers.
XII	—	4·66	17·02	—	9·69	—	—	
XIII	—	6·68	23·21	—	13·1	—	—	

* The two experiments in which this solution was fungicidal were carried out on April 29 and May 11; the same dilution used on May 18, 24 and 28 proved to be not quite fungicidal.

of precipitated sulphur, a quantity of Solution XI was diluted with a known volume of water and the correct amount of weak hydrochloric acid added to neutralise the liquid and precipitate the polysulphide sulphur. This liquid was then made up to the same volume as that of the diluted unaltered Solution XI. The liquid containing the very finely precipitated sulphur was well shaken before being used and was sprayed on mildewed leaves at the same nodes as leaves sprayed with the clear No. XI solution. Both liquids were found to be equally fungicidal. This experiment was repeated and the same result was obtained. Although this suggests that the precipitated sulphur has some action on the fungus it does not afford satisfactory evidence that the precipitated sulphur alone was responsible for the fungicidal action because ammonium chloride was present in the liquid after precipitation. This amount of ammonium chloride, namely, 0·28 %, has been found to have a severe checking action on the fungus. (See Table 4.) Further work in this direction is in progress.

SUMMARY.

1. The resistance of the hop-mildew (*Sphaerotheca Humuli* (DC.) Burr.) to polysulphide solutions varies according to its stage of development. The earliest stages after infection and antecedent to the production of conidiophores are the most resistant, requiring approximately a solution of double the concentration lethal for the "powdery" conidial stage. The "powdery" conidial stage occurring on young leaves is more resistant than the same stage on older leaves.
2. Conclusive proof has been obtained that with polysulphide solutions neither the total sulphur content nor the sulphide sulphur content gives an index of their fungicidal value.
3. The percentage of polysulphide sulphur in polysulphide solutions appears to be the factor determining their fungicidal value.
4. The fungicidal value of such solutions does not depend upon the nature of the polysulphides present.

(*Received March 6th*, 1919.)

THE AMOUNT AND COMPOSITION OF RAIN FALLING AT ROTHAMSTED.

(BASED ON ANALYSES MADE BY THE LATE NORMAN H. J. MILLER.)

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THE composition of rainwater was a matter of serious interest to the agricultural chemists of the last generation. It was a side issue of the great controversy on the source of nitrogen for plant life, which occupied much time and energy between 1840 and 1870. The main issue was whether plants could or could not assimilate free nitrogen from the air; this was settled in the case of non-leguminous crops by the investigations of Lawes, Gilbert and Pugh in 1861[1]. The side issue was whether the nitrate and ammonia necessary for vegetation would need to be supplied by fertilisers or whether the natural stores in the rain and the air would suffice.

Liebig had stated[2]:

"If the mineral elements, phosphates, etc., be duly supplied, the plant will obtain a sufficient supply of ammonia from the atmosphere": and again:

"If the soil be suitable, if it contains a sufficient quantity of alkalis, phosphates and sulphates, nothing will be wanting. The plants will derive their ammonia from the atmosphere as they do carbonic acid[3]."

Liebig clearly supposed that there was a considerable amount of nitrogen in the rain, and while he does not seem to have committed himself to any figure in his earlier writings, he published in 1863[4] an estimate of 24 lb. of nitrogen per acre per annum.

Lawes and Gilbert did not accept this position. They showed by field experiments that the crop yield is proportional to the ammonia

[1] *Phil. Trans.*, 1861, Part II, 431.

[2] *Letters on Chemistry*, 1851, 3rd ed., 519. In this Letter, the 34th, Liebig sets out his views with characteristic clearness.

[3] *Farmers' Magazine*, 1847, 16, 511.

[4] *Natural Laws of Husbandry*, 1863, 290.

supplied in the manure[1]. Further, they analysed the rain falling at Rothamsted, and their results indicated that it supplied only about 5 lb. per acre of ammonia[2], a quantity far below the 50 lb. of nitrogen needed by a 32 bushel crop of wheat. Their analytical procedure was extremely laborious, the Nessler test not then having been devised; in some experiments it even involved the distillation of over two hundredweights of rain, and evaporation of the distillate with sulphuric acid. Lawes and Gilbert had not been the first in the field; they acknowledged their indebtedness to Boussingault who, working on his experimental farm at Bechelbronn in Alsace, with simpler methods and much smaller quantities of rain, had obtained results very similar to their own[3].

Lawes and Gilbert were unable to make satisfactory determinations of the nitric nitrogen in the rain although they recognised its presence: further analyses were therefore made in the two following years, 1855 and 1856, but the work was not done in the Rothamsted laboratories, Gilbert being too fully occupied with the field plots; it was carried out by J. T. Way, then a promising young agricultural chemist. His figures also were far lower than the crop requirements, being in 1855 only 6·5 lb. per acre, and in 1856 8·0 lb. per acre for the sum of ammonia[4] and nitrate. The regular determinations were then discontinued, but numbers of occasional analyses were made, first by Edward Frankland and afterwards by Warington. These results were quite consistent with the measurements of 1855 and 1856, and lent no support to Liebig's view.

The subject would perhaps have been allowed to drop, but for the circumstance that Lawes and Gilbert in 1870 erected the famous drain gauges at Rothamsted and proceeded to make determinations of the amount of nitrate and ammonia percolating through them. This necessitated systematic analyses of the whole of the rainfall and of the drainage water over a period of years. Lawes and Gilbert were fortunate in the men to whom they entrusted the undertaking. The analytical work was done between 1877 and 1885 by the late Robert Warington, and from January, 1888, to 1916 by the late N. H. J. Miller, while the

[1] For their side of this controversy see J. B. Lawes and J. H. Gilbert, "On Agricultural Chemistry," *Journ. Roy. Agric. Soc.*, 1851, **12**, 1.

[2] *British Assoc. Reports*, 1854. As will be shown later on, even this figure is twice what it should be.

[3] Now that our French friends have recovered Bechelbronn we hope it will be found possible to commemorate in some adequate manner the important work carried out on this farm by Boussingault in laying the foundations of modern agricultural chemistry.

[4] *Journ. Roy Agric. Soc.*, 1856, **17**, 123, 618.

collection of samples at the gauges was done by E. Grey, who happily is still continuing the same work. Warington and Miller differed widely in personal characteristics but they were equally reliable analysts and equally undeterred by the monotony of the routine involved in the systematic work. Miller indeed made it his life-work, and only his sudden and unexpected death in January, 1917, prevented the completion of an important monograph which he had in preparation on the composition of rain and drainage waters.

Miller's results show that the data of 1855 and 1856, which had been wholly inadequate to give any support to Liebig's view, were probably in excess of the actual facts; the ammoniacal nitrogen apparently having been over estimated and given as 6 or 7 lb. per acre instead of 2·5 lb. to 3 lb.; during Miller's period the sum of the ammoniacal and nitric nitrogen was only 4 lb. per acre,—an amount still less adequate to supply the needs of crops.

Miller published his first paper in 1905[1], but since then a further ten years' results have accumulated which it is proposed to discuss here. The work has now been modified: no useful agricultural purpose would be served by continuing it in its original form, and its interest now lies in its relationship to atmospheric pollution. For the Rothamsted rain is collected in a part of the country which is fairly free from sources of impurity, and it may be taken as typical of "pure" rain: it thus affords a basis for estimating the amount of pollution. From this point of view there is the possibility of a useful continuation of the work.

The amount and distribution of the rainfall at Rothamsted.

The rainfall records at Rothamsted extend continuously over a period of 66 years, the results being

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	
Average for 60 years (Sept. 1853–Aug. 1912)	2·37	3·18	2·58	2·47	2·34	1·81	
Average for 28 years (Sept. 1888–Aug. 1916)	1·87	3·23	2·64	3·05	2·15	2·02	
	March	**April**	**May**	**June**	**July**	**Aug.**	**Total**
Average for 60 years (Sept. 1853–Aug. 1912)	1·92	1·84	2·19	2·45	2·50	2·69	28·34
Average for 28 years (Sept. 1888–Aug. 1916)	2·41	1·69	1·99	2·53	2·41	2·83	28·82

The average for the year over the whole period is 28·3 inches. It will be shown later, however (p. 324), that the rainfall tends to increase:

[1] This *Journal*, 1905, 1, 280–303.

for the last 14 years the average for the year has been 29·98 inches, and but for three abnormally dry years (1905–6, 1908-9, 1913–14) would have been well over 30 inches. Rothamsted lies in the rather narrow wet strip that runs in a north-easterly direction across the eastern counties, and separates the dry region, including the Thames Valley, South Middlesex, East Berkshire and East Oxfordshire on the south, from another dry tract comprising Bedfordshire, Cambridgeshire, Huntingdon, East Northamptonshire, etc., on the north.

The distribution calls for little comment. The four wettest months are July, August, October and November, and the four driest are February[1], March, April and June: allowing for the varying number of days, April is the driest month of all; this is usual over one-half the area of the British Isles[2]. Further details are given in Table 1, p. 331.

The amount and distribution of nitrogen compounds in the rain.

The amounts of ammoniacal and nitric nitrogen present in the rain are given in Table 2 (p. 332): in lbs. per acre they are

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Nitrogen as Ammonia (NH_3)	0·217	0·235	0·219	0·223	0·182	0·159
Nitrogen as Nitrate...	0·103	0·161	0·111	0·115	0·087	0·088
Sum ...	0·320	0·396	0·330	0·338	0·269	0·247
Rainfall ...	1·87	3·23	2·64	3·05	2·15	2·02

	March	April	May	June	July	Aug.	Total
Nitrogen as Ammonia (NH_3)	0·205	0·201	0·229	0·245	0·249	0·280	2·644
Nitrogen as Nitrate...	0·098	0·094	0·114	0·114	0·111	0·131	1·327
Sum ...	0·303	0·295	0·343	0·359	0·360	0·411	3·971
Rainfall ...	2·41	1·69	1·99	2·53	2·41	2·83	28·82

The yearly fluctuations in ammoniacal nitrogen are shown in Fig. 1: they are seen to follow the rainfall very closely with only four exceptions: the year 1893-4 when the ammonia fell although the rainfall rose, and 1895–6, 1901-2 and 1908-9, when the ammonia rose although there was less rain. Fig. 2 shows the fluctuations month by month; these also follow the rainfall. Tables 3 and 4 (pp. 333-4) give fuller details.

[1] February is one of the driest months in the whole year so far as actual rainfall is concerned, but there is a popular tradition which is still carried on by popular writers, and which no amount of statistical data is able to break, that it is a wet month. Probably the reason for the tradition is the old proverb "February fill dyke."

[2] H. R. Mill and C. Salter, *J. Roy. Met. Soc.*, 1915, **41**, 14.

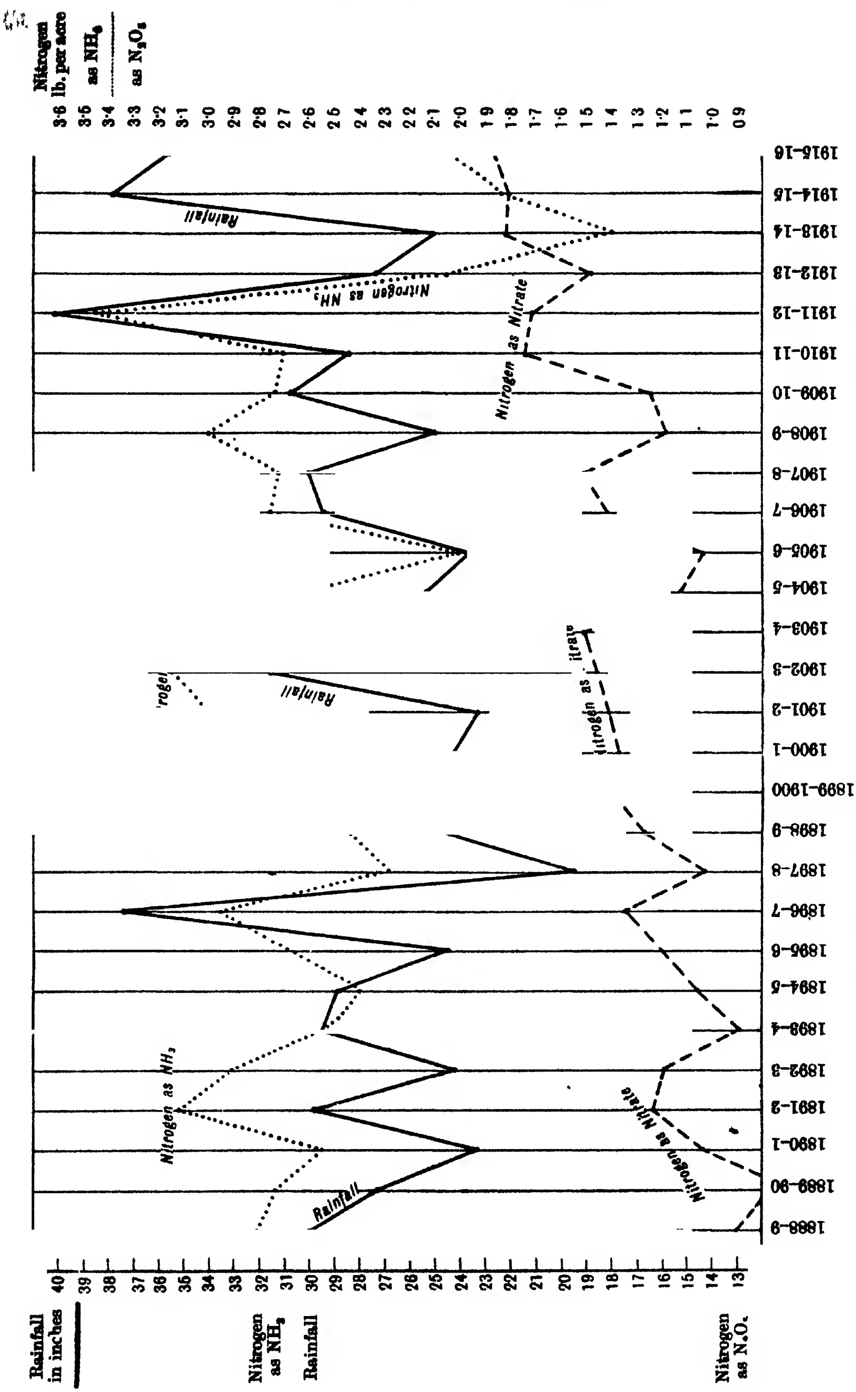


Fig. 1. Annual fluctuations in amounts of ammoniacal and nitric nitrogen in rainwater, 1888–1916.

Fig. 2, however, brings out the interesting point that the ammonia content of the rain is highest during May, June, July and August, and lowest during January, February, March and April.

The significance of this close relationship between rainfall and ammonia content is discussed later.

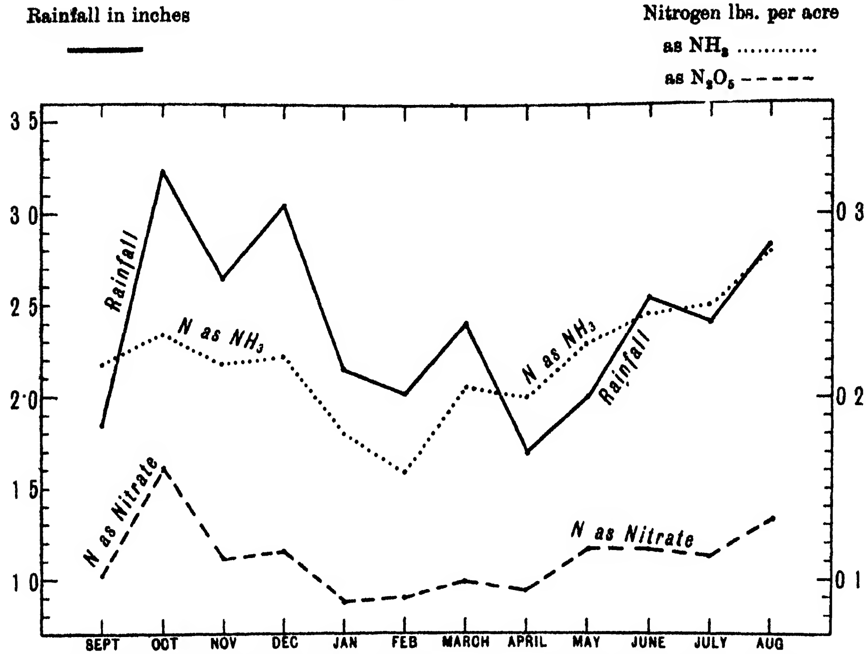


Fig. 2. Monthly fluctuations in amounts of ammoniacal and nitric nitrogen in rainwater, Sept. 1, 1888–Aug. 31, 1916.

The amount of nitrate in the rain.

The quantity of nitrate in the rain is shown in Tables 2, 3 and 5 (pp. 332–4); over the whole period it is half that of ammonia and amounts on an average to about one-tenth of a pound per acre per month (0·11 lb. per acre to be exact), but it varies less than the ammonia. In the Southern Hemisphere the proportions are reversed: at Lincoln, New Zealand, the ammonia is much smaller in amount than the nitrate[1], and at Melbourne the nitrate is high in amount and fluctuates[2]; in the Tropics also there is a considerable excess of nitrate over ammonia[3].

[1] G. Gray, *Proc. Austral. Assoc.*, Sydney, 1888.

[2] V. G. Anderson, *Journ. Roy. Meteorol. Soc.*, 1915, **41**, 99.

[3] J. B. Harrison and J. Williams, *Journ. Amer. Chem. Soc.*, 1897, **19**, 1.

Anderson[1] has argued that the values for nitric nitrogen are a function of the weather type which they may therefore help to characterise.

At Rothamsted the rain falling during the months May, June, July, August and October is richer in nitrate than that falling in January, February, March and April. This is similar to the distribution of ammonia. Examination of the curves of Fig. 1 shows that the nitric nitrogen fluctuated with the rainfall in much the same way as the ammoniacal nitrogen until 1910, but since then there has been no simple relationship. The monthly fluctuations shown in Fig. 2, also follow the rainfall and the ammonia.

For most of the period the relationship between ammoniacal and nitric nitrogen has been close and was almost exactly 2 : 1. Since 1912, however, the nitric nitrogen has increased so that on the four year average the nitric nitrogen is equal to the ammoniacal nitrogen, instead of being only half as much. This is not the result of a general rise throughout the whole of the year, but of a few exceptionally high values in the months February, March, July, August and October. During this period it happened that the rainfall in February and March has been unusually high; there was also one very wet July.

Ammoniacal and nitric nitrogen in rain: four year periods.

Four years' period {Sept. / Oct.	1888–1892	1892–1896	1896–1900	1900–1904	1904–1908	1908–1912	1912–1916
Nitric nitrogen ..	0·98	1·08	1·26	1·43	1·28	1·48	1·75
Ammoniacal nitrogen	2·82	2·63	2·68	2·98	2·54	2·97	1·84
Sum ...	3·80	3·71	3·94	4·41	3·82	4·45	3·59
Ratio: $\frac{\text{Ammoniacal}}{\text{Nitric}}$ nitrogen	3 : 1	5 : 2	2 : 1	2 : 1	2 : 1	2 : 1	1 : 1

The increase is presumably due to some artificial cause, though it is not easy to say what. During the period 1886 to 1908, which saw an enormous expansion of London into the Home Counties and a pushing out both of industrial concerns and of private residences, there was only a small rise in nitrate and none in ammonia. It is possible that the changes in stoves and gas-burners, which had become marked by 1912, and which have reduced the frequency of London fogs, have also tended to increase the nitrous fumes rather than the ammonia in the atmosphere.

The rain coming from the Atlantic contains much less nitrate than that falling in inland stations (see p. 317).

[1] V G Anderson, *Journ. Roy. Meteorol Soc.*, 1915, **41**, 99

Nitrites in rainwater.

The amount of nitrite in the Rothamsted rain has not been separately estimated: it is included in the nitrate figure. The rain from the Hebrides was frequently tested during the winter of 1911–12 and more frequently gave negative than positive results. Rain collected on land, however, usually contains nitrites and a long series of determinations made before the War at Prince Troubetzkoy's Experiment Station at Ploty in South Russia showed a considerable excess of nitrites in winter compared with summer rains.

The total nitrogen in rainwater.

No further determinations of organic nitrogen have been made beyond those recorded in Dr Miller's paper. The figure there given is 1·35 lb. per acre per annum; assuming this to be still correct on an average of the whole period the rain contained

2·64 lb. ammoniacal nitrogen,
1·33 ,, nitric nitrogen,
1·35 ,, organic nitrogen.

The ratios are almost exactly

2 parts ammoniacal nitrogen,
1 part nitric nitrogen,
1 part organic nitrogen.

But, as already pointed out, the proportions between ammoniacal and nitric nitrogen have fallen recently.

The sources of the combined nitrogen in rain.

Sources of ammonia. Certain indications as to the source of the ammoniacal nitric nitrogen in the air are afforded by their close relationship with the amount of rain: when the rainfall is high the ammonia is high, and conversely when the rainfall is low the ammonia is low also. There appear to be two possible sources: the ammonia may be brought into the district with the rain, or it may be already present in the atmosphere and dissolved out by the rain: in the latter case a constant renewal of the atmospheric supply has to be assumed, otherwise the first rain would wash out all that was present, leaving nothing to be removed later.

If the ammonia came with the rain the whole phenomena would be readily explicable: the more the rain the greater the amount of ammonia.

Since the larger part of the rain comes from the Atlantic it would follow that most of the ammonia would come from there also.

This view was held by Boussingault and developed by Schloesing[1] in an important series of papers. It has been subjected to a critical examination at Rothamsted.

Arrangements were made for the systematic collection of samples of rain at various stations (usually lighthouses) in the Hebrides and in Iceland, remote from atmospheric pollution. These samples were then sent to Rothamsted for analysis.

The results were[2]

		Nitrogen				
		Per million		Lbs. per acre per annum		
	Rainfall Inches	As Ammonia Average	As Nitrates Average	As Ammonia	As Nitrates	Sum
Rothamsted	28·04	0·437	0·202	2·774	1·251	4·025
Laudale, Ardgour ...	88·80	0·138	0·063	2·784	1·260	4·044
Barrahead, Berneray ...	35·28	0·145	0·138	1·164	1·104	2·268
Shilay Monach Islands, N. Uist.	48·36	0·115	0·054	1·260	0·588	1·848
Butt of Lewis, Stornoway	41·19	0·039	0·033	0·361	0·305	0·666
Vifilsstadir, Iceland ...	38·34	0·091	0·030	0·802	0·263	1·065

Much less ammonia was found than at inland stations and it was not always certain that the sample collected was as pure as the rain. In spite of serious efforts there was considerable difficulty at the lighthouses in keeping the rain gauges free from bird droppings, and it is possible that some of the ammonia came from adventitious contamination.

No analyses, so far as we know, have been made of rainwater collected on the Atlantic itself. One of us (E. J. R.) made several attempts to secure good samples when crossing and recrossing in 1909 and again in 1912, but without success. But even if the atmosphere over the Atlantic is not entirely free from ammonia, it certainly contains considerably less than that over the land. We therefore cannot agree with Schloesing that the bulk of the ammonia comes from the sea, though possibly part of it does.

It seems necessary therefore to suppose that some, if not all, of the ammonia is derived from the atmosphere. Owing to its high solubility

[1] *Compt. Rend.*, 1875, **80**, 175. "Sur l'ammoniaque de l'atmosphère." *Ibid.*, **81**, 81 and 1252; 1876, **82**, 747, 846 and 969.

[2] N. H. J. Miller, *Journ. Scottish Meteorol. Soc.*, 1913, iii, **16**, 141.

the ammonia may be expected to dissolve freely, in which case constant renewal would be necessary in order to account for the fact that high rainfall brings down more ammonia than low rainfall.

This constant renewal necessitates contact with a source of ammonia which, moreover, gives up its ammonia uniformly so that a definite equilibrium is attained. As soon as a shower of rain has fallen and removed the ammonia from the atmosphere a further supply must be drawn from the source sufficient to restore the disturbed equilibrium. The next shower removes some of this, but again the equilibrium is restored. On this assumption the total quantity brought down by the rain in any year would depend on the amount of the rainfall, but the quantity per inch of rain would show less variation.

Three possible sources have been considered: the sea, which has already been discussed, the soil, and the atmosphere over large towns and cities.

The soil seems quite a likely source: changes are constantly occurring there with formation of ammonia which is then transformed to nitrate. The amount of ammoniacal nitrogen existing as such at any time is at least 5 lb. per acre in the top 9 in., and its rate of diffusion into the atmosphere might be fairly rapid.

Hall and Miller[1] endeavoured to obtain information as to this possibility by exposing shallow dishes of sulphuric acid, some close to the ground and others four feet above it, and then determining the ammonia absorbed by the end of each month: the experiment lasted for two years. The amounts absorbed corresponded only to 0·99 and 1·28 lb. per acre in the respective years,—much less than those recorded by previous observers, perhaps because of the efficiency with which dust and insects were excluded: the lower dishes, however, did not usually contain more ammonia than the upper ones, excepting when sulphate of ammonia was applied to the soil. No conclusions could be drawn as to whether the soil normally gives up ammonia to the atmosphere, or whether it absorbs ammonia from the atmosphere. From the circumstance that soil gives up ammonia when dressed with sulphate of ammonia it seems legitimate to infer that some ammonia is continuously being evolved.

The seasonal fluctuations in the amount of ammonia in the rain are quite consistent with the view that the ammonia comes from the soil. The amount of ammoniacal nitrogen in the rain is lowest in the four months, January, February, March and April, when biochemical

[1] This *Journal*, 1911, **4**, 46.

activity in the soil is at a minimum; and highest in June, July, August and October, when biochemical activity in the soil is at a maximum.

The other source with which the atmosphere may be in equilibrium is the atmosphere of cities, where the amount of ammonia is markedly greater than in the country. The amount of ammonia brought down by the rain of certain towns is as follows, the Rothamsted and Malvern figures for the same periods being given for purposes of comparison:

Nitrogen as ammonia in lbs. per acre per annum[1].

Average of four years, 1914–18	Summer	Winter	Total
London (Embankment Gardens)	5·22	5·04	10·26
Newcastle-on-Tyne	4·92	6·06	10·98
Malvern	1·14	0·48	1·62
Rothamsted (1912–1916) ...	1·03	0·81	1·84

If the whole of the ammonia were carried down in the rain there would of course be none to travel out into the country, but as pollution is constantly going on there may be an excess during the intervening dry periods. It is not certain, however, that atmospheric pollution would be sufficient by itself to account for all the ammonia present in the rain. The value obtained at Rothamsted is not likely to be higher, indeed it is probably lower, than the average over Great Britain: assuming only 2·6 lb. per acre the total amount of ammonia brought down on the 56,000,000 acres of Great Britain would be 65,000 tons of combined nitrogen, equivalent to 325,000 tons of sulphate of ammonia per annum[2]. The total coal consumption of the country is approximately 200,000,000 tons per annum: if all this were handled at gas works or in coke ovens it would yield some 2,000,000 tons of sulphate of ammonia, assuming 22·7 lb. sulphate of ammonia were obtained from each ton of coal. But the open grate is far less efficient as a producer of ammonia than the gas retort, and nothing approaching this quantity is likely to arise. Of the amount actually formed no less than 400,000 tons is collected from gas and recovery plants, and a further large quantity is absorbed in soot. The amount discharged into the atmosphere is the difference between these quantities and the total production: it is impossible to make a satisfactory estimate, but there seems hardly enough left to furnish even the 325,000 tons of sulphate of ammonia,

[1] Taken from reports of Advisory Committee on Atmospheric Pollution.

[2] This figure is rather interesting: it is nearly 900 tons per day, and is considerably more than the whole of the artificial nitrogenous fertilisers used by farmers in the United Kingdom.

which, as we have seen, is probably a minimum value for the total brought down in the rain.

There is a further difficulty in the assumption that atmospheric pollution is the main source of the ammoniacal nitrogen of country air. Atmospheric pollution of cities is as bad in winter as in summer if not worse, so that if it were the source of most of the ammonia in country rain we should expect at least as much in winter as in summer: in point of fact there is less. And, moreover, so far as Rothamsted is concerned, the only town of any size to the south-west is Reading, but as this is more than forty miles away, it is hardly likely to serve as a sufficient reservoir.

The formation of nitrates or nitrous fumes in the air is commonly attributed to the major electrical discharges,—thunderstorms, etc. Berthelot has shown that the silent electric discharge causes a production of nitric acid from moist nitrogen and oxygen[1]. There is also the possibility that the electrical stresses in the atmosphere may have some effect; Chree[2] estimates these at about 200 to 300 volts per metre at ground level, and much more at higher levels: at the top of tall trees there may be 5000 volts per metre. The potential gradient required to make a spark pass in ordinary air at normal pressure is of the order of 30,000 volts per centimetre.

It has further been suggested by Soddy[3] that nitrous oxide may be formed by the action of the radium emanations always present in the lower portions of the atmosphere.

There are, however, other possible sources. Dust invariably contains nitrates, and in summer the atmosphere contains more dust than in the winter: the rain as we have seen also contains more nitrate. Gas flames and fires also produce nitrous fumes.

The close relationship between the amounts of ammoniacal nitrogen and of nitric nitrogen in the rain throws important light on the origin of the nitric compound. It must either be formed from the ammonia or come from the same source. It is possible, but not easy, to conceive of nitric oxides or acid compounds coming from the sea; it is not difficult to conceive of such compounds coming from the soil and from the air of cities. Formation from ammonia would present no serious difficulty: nitric compounds might arise from the oxidation of ammonia under the influence of the minor electrical disturbances and electrical stresses in the

[1] *Compt. Rend.*, 1906, **142**, 1367.
[2] *Journ. Roy. Meteorol. Soc.*, 1915, **41**, 121.
[3] *Chemical Soc. Reports*, 1911, **8**, 299.

atmosphere. It is known[1] that this oxidation proceeds under the action of the silent electric discharge, and it may well form part of the normal atmospheric phenomena.

In reviewing the evidence for and against these various possibilities one is driven to conclude that the ammonia in country rain probably arises from several sources and not from one only. The sea, the soil, and towns and cities may each contribute their share. Of these the soil appears to be the most important in view of the fact that the amount brought down by the rain is at a maximum in the summer months when biochemical activity in the soil is at its highest, and at a minimum in winter when biochemical activity in the soil is low. This does not amount to proof of the origin of the ammonia because the higher ammoniacal content of summer rain is affected by another factor: the rain during the summer months is probably on the whole formed at higher levels than that falling during winter months, and, having a greater distance to travel, it would wash out the ammonia from a larger quantity of air than is possible in the case of winter rain. A further effect is discussed on p. 328.

A certain amount of atmospheric ammonia may also come from the towns: the reality of this source is indicated by the fact that town rain-water is considerably richer in ammonia than country rainwater. This is unlikely to be the chief source, as the ammonia in town rain is high in winter, whereas we have seen it is then at its lowest in country rain.

The nitric nitrogen also probably arises from several sources. In the early years of the work the amount of nitric nitrogen recorded was only one-third that of the ammoniacal nitrogen, but for the greater part of the period of the observations it was one-half. Of late years there have been some exceptionally high values, causing the nitric nitrogen to become equal to the ammoniacal nitrogen. These changes indicate an artificial rather than a natural origin for part of the nitrate. But it cannot all arise in this way, for it is widely distributed over the world. Some of it may arise by direct combination of nitrogen and oxygen during lightning and other major electrical disturbances. For the greater part the close correlation between the amounts of nitric nitrogen and of ammoniacal nitrogen indicates either a common origin, or a formation of nitric compounds from ammonia by the minor electrical disturbances and electrical stresses normally occurring in the atmosphere, or in some other way.

[1] See W. G. Mixter, *Amer. Journ. Sci.*, 1898 [iv], 6, 217–224.

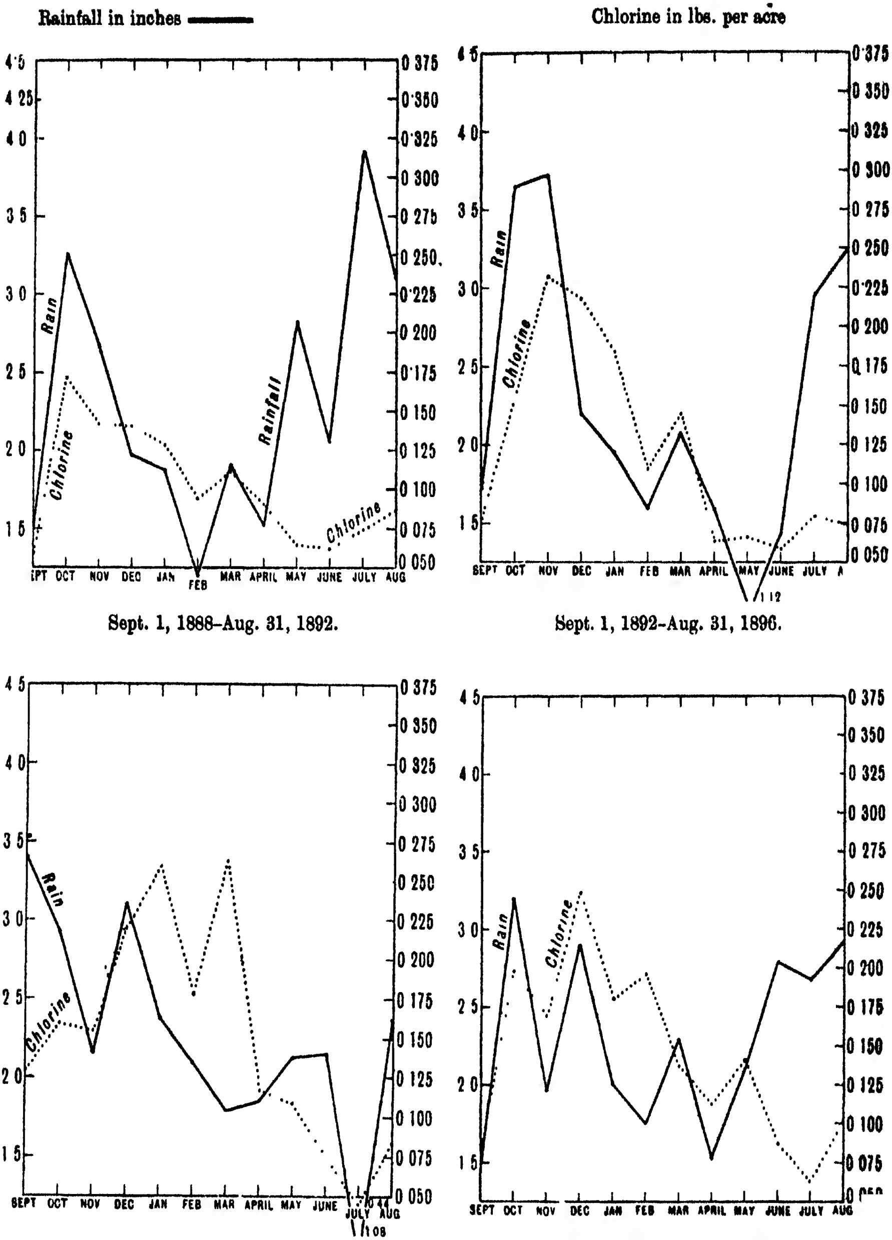
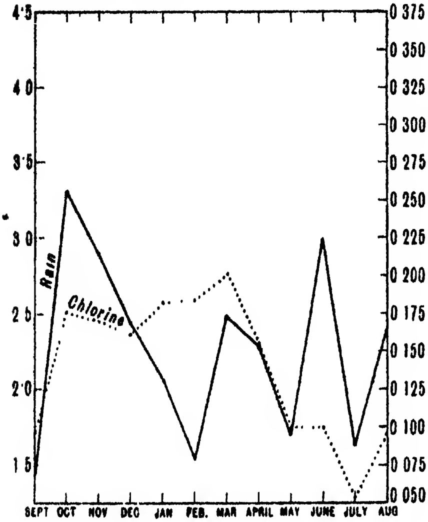
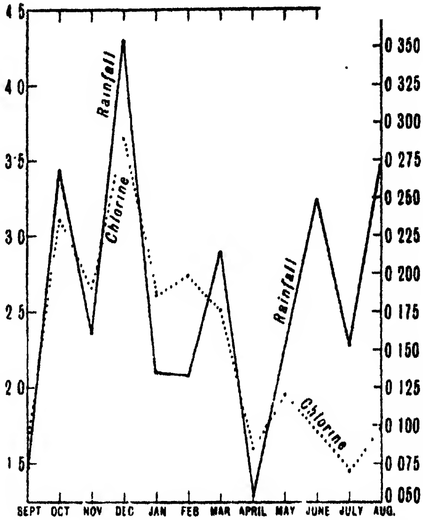


Fig. 3. Monthly fluctuations in amounts of chlorine in rainwater: four year periods between Sept. 1, 1888 and Aug. 31, 1916.

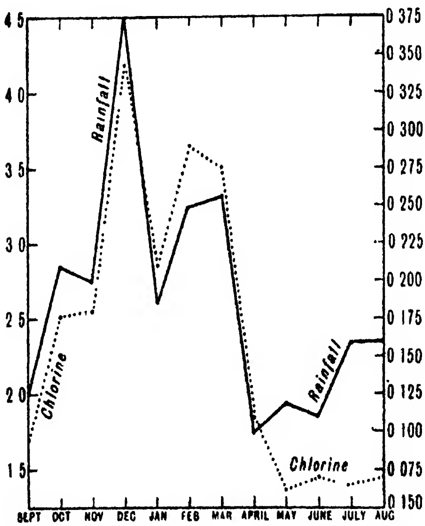
Rainfall in inches ——— Chlorine in lbs. per acre · · ·



Sept. 1, 1904–Aug. 31, 1908.



Sept. 1, 1908–Aug. 31, 1912.



Sept. 1, 1912–Aug. 31, 1916.

Fig. 3 (*cont.*).

The amount of chlorine in rainwater.

The chlorine is determined in the rainwater at Rothamsted because the values are wanted in connection with the drainage investigations. The data are given in Tables 6 and 7 (pp. 335-6): they throw interesting light on the origin of the rain.

The average amount of chlorine in the Rothamsted rainwater is 2·3 parts per million, bringing down 16 lb. per acre per annum, but the amount fluctuates considerably from year to year, the lowest being 10·3 lb. in 1889-90, and the highest 24·4 lb. in 1915–16. Most of this is brought down during the months September to April: a much smaller amount falls in the summer months. The fluctuations in the amount of chlorine carried down per acre closely follow the fluctuations in the amount of rainfall, especially in the months September to April: during the summer months, May to August, the rain contains so little chlorine that the fluctuations have less significance. This is well brought out by the curves (Fig. 3); only in two periods 1896-1900 and 1904-8, is there notable deviation from the close relationship in the winter months.

It has been usual to attribute the chlorine to sea spray blown over the land. This view is consistent with the facts, and would explain the large amount of chlorine in winter rain and the small amount in summer rain when gales are less common.

Another source exists, however. Some of the chlorine may come from fires: it is present in coal, which contains about 4 lb. per ton: it would be liberated during combustion in the gaseous form or as volatile chlorides. This source would be most in evidence during the winter.

The change in composition of Rothamsted rainwater.

Looking over the figures for successive four year periods given in Table 9 (p. 337) there seem to be signs of change in the composition of rain. The chlorine is increasing in amount, so also is the nitrate; the ammonia tends to fall, so that there is no steady alteration in the sum of the ammoniacal and nitric nitrogen. It is more easy to suggest possible explanations than to decide between them. The rainfall has increased since 1908, and this tends to increase the quantity of substances brought down per acre. The increase in rainfall has been especially marked in the winter months: we have no wind records at Rothamsted, but it is possible that the higher winter rainfall has been accompanied by heavier gales carrying more chlorine from sea spray. The increase in

amount of chlorine is not accidental: it is equally manifest in the Cirencester data:

	Rothamsted		Cirencester (Kinch)[1]	
	Rain (inches per annum)	Chlorine (lbs. per acre per annum)	Rain (inches per annum)	Chlorine (lbs. per acre per annum)
1888–89 to 1891–92	27·65	12·25	27·99	14·53
1892–93 to 1895–96	27·95	14·35	26·14	16·36
·1896–97 to 1899–1900	28·12	17·90	27·26	17·33
1900–01 to 1903–04	27·58	17·23	31·83	24·78
1904–05 to 1907–08	27·14	16·75	26·22	17·60
1908–09 to 1911–12	31·03	18·48	31·38	22·43
1912–13 and 1913–14	26·17	15·89	30·49	26·12

There appears, however, to have been some other factor concerned besides the increase in rainfall, as the concentration of chlorine in the rainwater has also increased: formerly the rain contained about 2–2½ parts of chlorine per million, now the amount varies from 2½–3 parts per million.

The increase in amount of nitric nitrogen is shown in Tables 5 and 9 and in Fig. 4: it is not as steady as that of chlorine and is intensified by a few abnormally high values especially in two dry months February and July, 1913, in the wet October, 1913, in the dry May, 1914, and in the wet August, 1916. It is, of course, impossible now to check these figures: we have gone carefully through the laboratory notes and can find nothing to indicate that they are unreliable. Even apart from these exceptional cases, however, there is a clear upward trend in the nitrate figures. Examination of the curves in detail shows that the tendency to increase is spread over the whole year and is not confined to any one season: formerly there was about 0·12 to 0·19 parts of nitric nitrogen per million of rain, now the figures are 0·2 to 0·3 parts.

Although the rise in nitric nitrogen is similar to that of chlorine the phenomena are not necessarily related. We have no grounds for supposing that sea spray contains nitrates in sufficient quantity to produce the observed effects. On the other hand if some of the chlorine comes from coal a relationship might be expected, as part of the nitrate may come from the same source.

The ammonia, on the other hand, has decreased in concentration: formerly there were usually 0·4 to 0·5 parts per million, now there are more usually 0·2 to 0·3 parts. This is shown in Tables 4 and 9 and in Fig. 4.

[1] Data collected from papers in *Agricultural Students' Gazette* and *Trans. Chem. Soc.*, 1887, **51**, 92 and 1900, **77**, 1271.

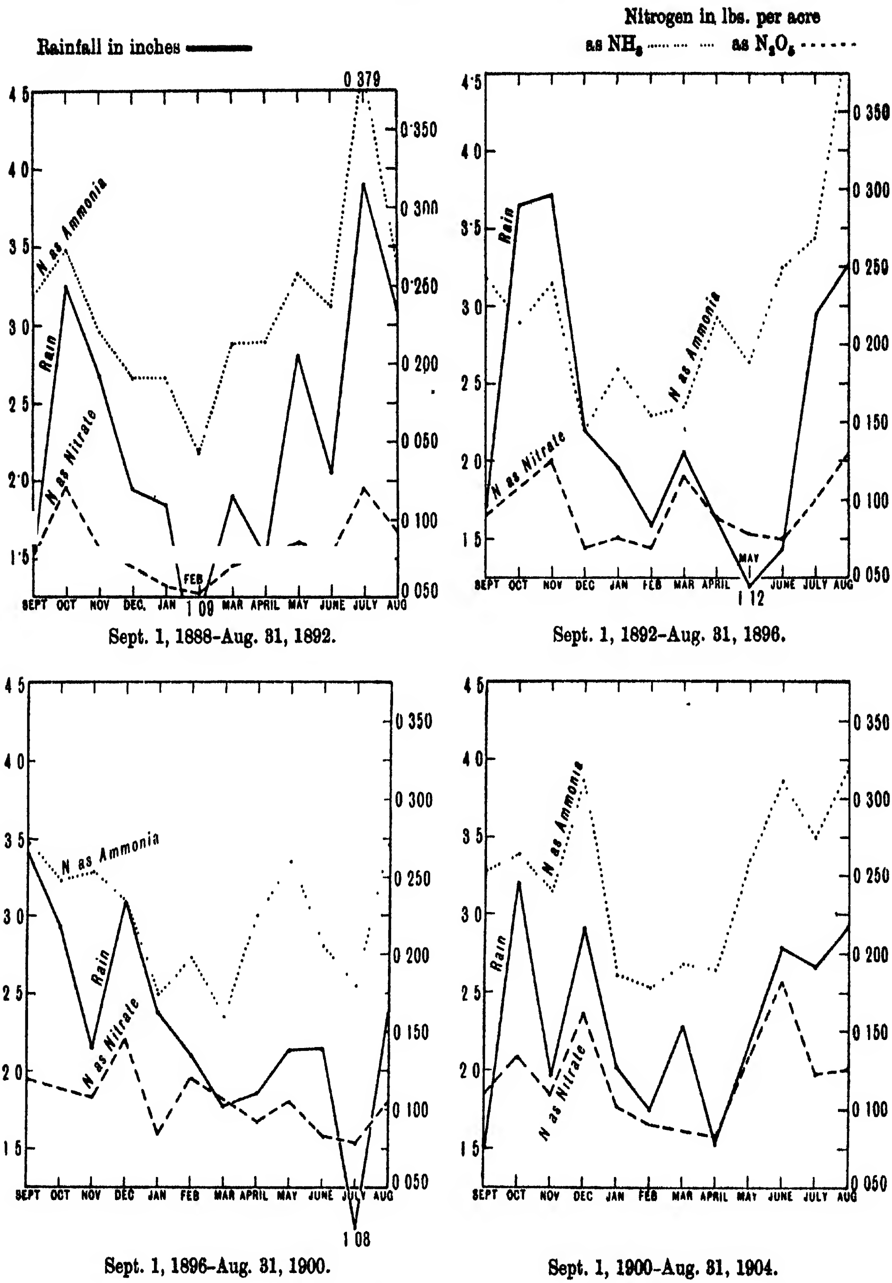
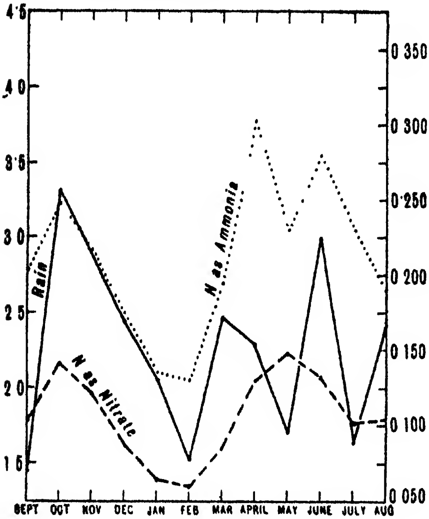


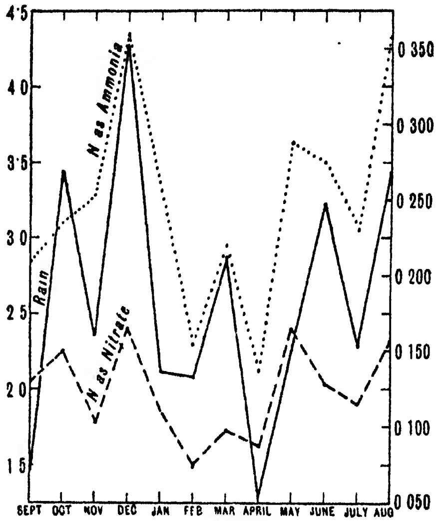
Fig. 4. Monthly fluctuations in amounts of nitric and ammoniacal nitrogen in rainwater: four year periods between Sept. 1, 1888 and Aug. 31, 1916.

Nitrogen in lbs. per acre
as NH_3 ·········· as N_2O_5 - - -

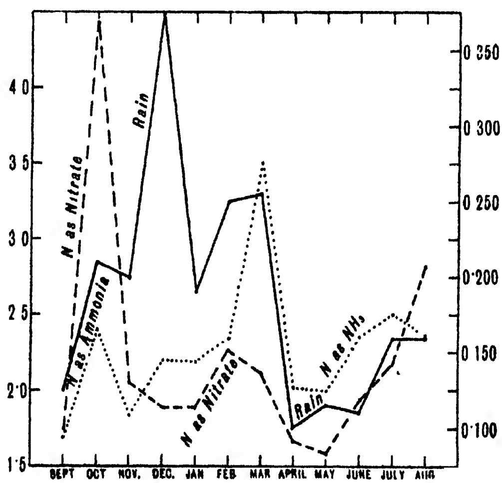
Rainfall in inches ———



Sept. 1, 1904–Aug. 31, 1908.



Sept. 1, 1908–Aug. 31, 1912.



Sept. 1, 1912–Aug. 31, 1916.

Fig. 4 (*cont.*).

The fall in ammonia and the simultaneous rise in nitrate may of course be wholly unconnected, but it suggests that a former source of ammonia now turns out nitrate instead, or that more nitrate is being produced from ammonia in the air than was previously the case. We have no data for examining the second possibility, but the first has some degree of probability: modern incandescent burners, gas and coal fires, may discharge more nitrous fumes and less ammonia into the atmosphere than the older types.

Dissolved oxygen in rainwater.

During the year 1915 estimations of dissolved oxygen in rain at Rothamsted were made[1] on rainfalls exceeding 0·30 inch. The great majority of the samples were found to be nearly saturated with oxygen but a few were considerably under saturation, notably those collected

DISSOLVED OXYGEN IN RAINWATER. PARTS PER MILLION

Date 1918	Temp. in ° C. at time of collection	Bar. pressure mm.	Dittmar's figure for 760 mm.	Saturation corrected for pressure	Oxygen in sample	Percentage saturation (corrected)
			Summer Rain.			
July 11	15·5	745	10·2	9·8	9·7	99
„ 14	18·0	748	9·7	9·3	9·3	100
„ 19	20·5	750	9·2	8·8	8·7	99
„ 23	16·0	741	10·1	9·5	8·9	94
„ 26	15·5	749	10·2	9·9	9·1	92
Aug. 2	17·0	745	9·9	9·5	8·8	93
„ 25	18·0	749	9·7	9·3	8·5	91
				Mean	9·0	95 %
			Winter Rain.			
Nov. 29	9·5	752	11·6	11·2	10·6	95
Dec. 3	13·0	749	10·7	10·3	10·2	99
„ 10	11·0	750	11·2	10·8	10·6	98
„ 16	7·5	753	12·1	11·8	10·7	91
„ 19	7·0	733	12·2	11·7	11·8	101
„ 20	6·0	741	12·5	12·1	13·2	109
				Mean	11·2	99 %

in July and August. More recent determinations of summer and winter rains gave mean values of 95 and 99 per cent. respectively calculated on Dittmar's figures for distilled water saturated with air at the observed temperature and pressure. It seems possible that the greater height of

[1] This *Journal*, 1917, **8**, 331–337.

clouds in summer may account for the smaller oxygen content of rain, as condensation would occur at pressures much below normal. The velocity of the rain drops falling from greater heights may not allow time for equilibrium to be established with conditions at ground level.

The amounts of dissolved oxygen found were 9·0 parts per million in the summer, corresponding to 20·8 lb. per acre during the period May to August inclusive, and 11·2 parts per million in winter, corresponding to 26 lb. per acre during the four months November to February. Over the whole year the amount brought down was 66·4 lb. per acre. The results are shown in the table on p. 328.

The chemical characterisation of rainwater.

The above discussion shows that the rain falling at Rothamsted in winter differs chemically from that falling in summer: the values being

	Parts per million Four-month periods		Lbs. per acre Four-month periods	
	Winter (Nov.–Feb.)	Summer (May–Aug.)	Winter (Nov.–Feb.)	Summer (May–Aug.)
Ammoniacal nitrogen ...	0·35	0·45	0·78	1·00
Nitric nitrogen	0·18	0·21	0·40	0·47
Chlorine	3 38	1·38	7·50	3·08
Dissolved oxygen ...	11·2	9·0	26·0	20·8*

* In the whole year it is estimated that 66·4 lb. of dissolved oxygen is brought down.

The winter rainfall is richer in chlorine and oxygen but poorer in ammoniacal and nitric nitrogen than the summer rainfall.

The marked differences in the amounts of chlorine and ammonia suggest that winter rain may differ in origin from summer rain. The winter rain resembles Atlantic rain in its high chlorine and low ammonia content, suggesting that it is derived from the Atlantic. The summer rain, on the other hand, is characterised by a lower content of chlorine and higher proportion of ammonia which suggests that it arises from the soil by evaporation of water and condensation at higher altitudes than in the case of winter rain; this would also account for the difference in amount of dissolved oxygen.

SUMMARY.

The ammoniacal nitrogen in the Rothamsted rainwater amounts on an average to 0·405 part per million, corresponding to 2·64 lb. per acre per annum. The yearly fluctuations in lbs. per acre follow the rainfall fairly closely. The monthly fluctuations also move in the same direction as the rain, but the general level is highest during May, June,

July and August, and lowest during January, February, March and April (Tables 1, 2, 3, and Figs. 1 and 2).

The nitric nitrogen is on an average one-half the ammoniacal, viz., 1·33 lb. per acre per annum. The amounts fluctuated year by year and month by month in the same way as the ammoniacal nitrogen and the rainfall until 1910, since when there has been no simple relationship (Tables 1, 2, 3, and Figs. 1 and 2).

Reasons are adduced for supposing that the ammonia arises from several sources. The sea, the soil, and city pollution may all contribute. Neither the sea nor city pollution seems able to account for all the phenomena: the soil is indicated as an important source by the fact that the ammonia content is high during periods of high biochemical activity in the soil, and low during periods of low biochemical activity.

The close relationship between the amounts of ammoniacal and nitric nitrogen suggests either a common origin or the production of nitric compounds from ammonia.

The average amount of chlorine is 2·43 parts per million bringing down 16 lb. per acre per annum (Table 6). The fluctuations closely follow the rainfall both month by month and year by year, but the general level is much higher during the months September to April than during the summer months (Fig. 3). It seems probable that the chlorine comes from the sea, but some may come from fuel.

Since 1888, when the experiments began, to 1916, when they terminated, there has been a rise in the amounts of nitric nitrogen and of chlorine in the rain. (Tables 5, 7, 8 and 9; Figs. 3 and 4.) In the case of chlorine a parallel series of determinations made at Cirencester over the same period shows a similar rise (p. 325). There is no rise of ammonia but on the contrary a tendency to drop (Tables 4 and 9; Fig. 4): the sum of ammoniacal and nitric nitrogen shows little change over the period. This seems to suggest that a former source of ammonia is now turning out nitric acid: it is possible that modern gas burners and grates tend to the formation of nitric oxides rather than of ammonia.

Rain contains on an average 10 parts of dissolved oxygen per million, the amount being higher in winter than in summer: 66·4 lb. per acre per annum was brought down during the two years over which the determinations extended (p. 328).

The marked difference in composition between summer and winter rainfall suggests that these may differ in their origin. The winter rain resembles Atlantic rain in its high chlorine and low ammonia and nitrate content: the summer rain is characterised by low chlorine but

high ammonia and nitrate content, suggesting that it arises by evaporation of water from the soil and condensation at higher altitudes than in the case of winter rain.

TABLE 1. *Rainfall at Rothamsted during harvest years, Sept. 1–Aug. 31.*

(For previous years see this *Journal*, 1905, 1, 300.)

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July	August	Year
	inches	inches	inches	inches	inches	inches	inches	inches	inches	inches	inches	inches	inches
1905–6	2·25	1·67	3·23	1·10	4·09	2·33	1·64	0·80	1·38	3·61	0·42	1·25	23·77
1906–7	1·15	5·30	4·22	2·79	1·25	1·48	1·31	2·85	2·40	2·61	2·21	1·80	29·37
1907–8	0·78	4·89	2·44	3·40	1·58	1·34	3·40	3·28	1·89	1·68	2·43	3·01	30·12
1908–9	1·56	2·26	0·82	2·07	0·98	0·43	3·74	1·82	1·32	4·10	3·10	2·74	24·94
1909–10	1·94	5·19	1·25	3·42	2·18	3·78	1·24	1·58	2·16	2·81	2·00	3·38	30·93
1910–11	0·89	2·83	3·93	5·36	1·38	1·88	2·27	1·59	3·79	2·74	0·65	1·05	28·36
1911–12	1·52	3·47	3·44	6·26	3·89	2·21	4·29	0·17	1·47	3·28	3·35	6·53	39·88
1912–13	2·72	2·74	2·52	3·42	3·36	1·00	2·52	3·16	1·81	1·20	1·29	1·58	27·32
1913–14	1·50	3·49	2·94	0·87	0·88	3·45	4·43	1·15	1·42	1·72	1·57	1·59	25·01
1914–15	1·30	2·53	3·10	8·11	4·11	4·54	1·38	1·22	2·48	1·79	4·72	2·59	37·87
1915–16	2·49	2·60	2·38	5·56	2·24	3·97	4·92	1·43	1·97	2·71	1·77	3·58	35·62
Average for period Sept. 1, 1905–Aug. 31, 1916	1·65	3·36	2·75	3·85	2·36	2·40	2·83	1·73	2·01	2·57	2·14	2·64	30·29
Av. for whole period Sept. 1, 1888–Aug. 31, 1916	1·87	3·23	2·64	3·05	2·15	2·02	2·41	1·69	1·99	2·53	2·41	2·83	28·82

TABLE 2. *Nitrogen as ammonia and nitric acid in rainwater collected at Rothamsted.*

(For previous years see this *Journal*, 1905, 1, 282.)

Harvest year September 1 to August 31	Rainfall in inches	Nitrogen Per million As NH_3	Per million As N_2O_5	Per acre (lb.) As NH_3	Per acre (lb.) As N_2O_5	Per acre (lb.) Total	% of total As NH_3	% of total As N_2O_5
1901–2	23·26	0·571	0·267	3·006	1·407	4·413	68·1	31·9
1902–3	31·26	0·447	0·203	3·159	1·436	4·595	68·8	31·2
1903–4	31·50	0·424	0·214	3·026	1·523	4·549	66·5	33·5
1904–5	25·31	0·460	0·197	2·634	1·128	3·762	70·0	30·0
1905–6	23·77	0·373	0·194	2·007	1·045	3·052	65·8	34·2
1906–7	29·37	0·417	0·213	2·774	1·416	4·190	66·2	33·8
1907–8	30·12	0·404	0·222	2·752	1·516	4·268	64·5	35·5
1908–9	24·94	0·532	0·211	3·001	1·193	4·194	71·6	28·4
1909–10	30·93	0·393	0·179	2·753	1·254	4·007	68·7	31·3
1910–11	28·36	0·421	0·272	2·705	1·743	4·448	60·8	39·2
1911–12	39·88	0·381	0·190	3·436	1·718	5·154	66·7	33·3
1912–13	27·32	0·336	0·239	2·078	1·478	3·556	58·4	41·6
1913–14	25·01	0·246	0·323	1·395	1·827	3·222	43·3	56·7
1914–15	37·87	0·214	0·211	1·837	1·808	3·645	50·4	49·6
1915–16	35·62	0·253	0·232	2·037	1·874	3·911	52·1	47·9
Average for period Sept. 1, 1901–Aug. 31, 1916	29·63	0·384	0·222	2·573	1·491	4·064	63·3	36·7
Average for whole period Sept. 1, 1888–Aug. 31, 1916	28·82	0·405	0·203	2·644	1·327	3·971	66·6	33·4

The analytical determinations are carried out once a month on a sample made up from the daily collections. After each wet day a sample is taken from the gauge proportional in amount to the rainfall: one decigallon per inch of rain: this is kept in a Winchester quart bottle. At the end of the month the whole sample is measured and should correspond with the total rainfall.

The average figures quoted in the tables are true averages of rainfall and lbs. per acre but not of parts per million. In the latter case the figure shows the number of parts which when multiplied by the average rainfall gives the average number of lbs. per acre. This is the old Rothamsted convention and has been in use so long that change would be a serious matter. The figures are usually not widely different from the true averages, but no definite relationship exists between them.

TABLE 3. *Average monthly amounts of nitrogen as ammonia and nitric acid in rainwater collected at Rothamsted.*

Sept. 1, 1888 to Aug. 31, 1916	Rainfall in inches	Nitrogen: Per million As NH_3	Per million As N_2O_5	Per acre (lb.) As NH_3	Per acre (lb.) As N_2O_5	Per acre (lb.) Total	% of total As NH_3	% of total As N_2O_5
September	1·87	0·514	0·244	0·217	0·103	0·320	67·8	32·2
October	3·23	0·321	0·220	0·235	0·161	0·396	59·3	40·7
November	2·64	0·366	0·186	0·219	0·111	0·330	66·4	33·6
December	3·05	0·323	0·167	0·223	0·115	0·338	66·0	34·0
January	2·15	0·374	0·179	0·182	0·087	0·269	67·7	32·3
February	2·02	0·349	0·193	0·159	0·088	0·247	64·4	35·6
March	2·41	0·376	0·180	0·205	0·098	0·303	67·7	32·3
April	1·69	0·524	0·245	0·201	0·094	0·295	68·1	31·9
May	1·99	0·508	0·253	0·229	0·114	0·343	66·8	33·2
June	2·53	0·428	0·199	0·245	0·114	0·359	68·2	31·8
July	2·41	0·457	0·204	0·249	0·111	0·360	69·2	30·8
August	2·83	0·438	0·205	0·280	0·131	0·411	68·1	31·9
Sept.–Dec.	10·79	0·366	0·201	0·894	0·490	1·384	64·6	35·4
Jan.–April	8·27	0·399	0·196	0·747	0·367	1·114	67·1	32·9
May–August	9·76	0·454	0·213	1·003	0·470	1·473	68·1	31·9
April–Sept.	13·32	0·471	0·221	1·421	0·667	2·088	68·0	32·0
Oct.–March	15·50	0·348	0·188	1·223	0·660	1·883	64·9	35·1
Whole year	28·82	0·405	0·203	2·644	1·327	3·971	66·6	33·4

TABLE 4. *Nitrogen as ammonia in rainwater collected at Rothamsted in parts per million.*

Harvest years, Sept. 1–*Aug.* 31.

(For previous results see this *Journal*, 1905, 1, 301.)

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July	August	Year
1905–6	0·269	0·300	0·350	0·781	0·150	0·250	0·350	0·800	0·625	0·300	2·125	0·425	0·373
1906–7	0·883	0·263	0·319	0·204	0·325	0·452	0·525	0·650	0·525	0·525	0·500	0·350	0·417
1907–8	1·083	0·375	0·344	0·200	0·625	0·400	0·375	0·375	0·500	0·656	0·367	0·333	0·404
1908–9	0·650	0·321	0·800	0·500	0·781	1·281	0·470	0·450	0·766	0·400	0·425	0·719	0·532
1909–10	0·917	0·263	0·550	0·250	0·350	0·225	0·300	0·625	0·450	0·475	0·425	0·400	0·393
1910–11	0·525	0·475	0·225	0·250	0·650	0·257	0·375	0·338	0·625	0·313	0·875	1·300	0·421
1911–12	0·300	0·200	0·650	0·500	0·550	0·375	0·213	0·317	0·500	0·313	0·400	0·250	0·381
1912–13	0·183	0·188	0·213	0·188	0·338	0·525	0·775	0·400	0·300	0 425	0·500	0·263	0·336
1913–14	0·217	0·225	0·058	0·263	0·300	0·158	0·150	0·250	0·575	0·400	0·600	0·650	0·246
1914–15	0·319	0·375	0·300	0·117	0·200	0·150	0·300	0·288	0·288	0·425	0·188	0·188	0·214
1915–16	0·167	0·350	0·117	0·133	0·142	0·263	0·375	0·225	0·350	0·325	0·350	0·263	0·253
Averages:													
1905–1916	0·433	0·289	0·320	0·260	0·351	0·274	0·365	0·438	0·468	0·398	0·428	0·390	0·355
1888–1916	0·514	0·321	0·366	0·323	0·374	0·349	0·376	0·524	0·508	0·428	0·457	0·438	0·405

TABLE 5. *Nitrogen as nitrates in rainwater collected at Rothamsted in parts per million.*

Harvest years, Sept. 1–*Aug.* 31.

(For previous results see this *Journal*, 1905, 1, 302.)

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July	August	Year
1905–6	0·188	0·192	0·225	0·325	0·083	0·133	0·083	0·450	0·400	0·175	0·475	0·213	0·194
1906–7	0·325	0·133	0·131	0·184	0·225	0·150	0·250	0·275	0·400	0·250	0·263	0·175	0·213
1907–8	0·625	0·213	0·213	0·092	0·225	0·213	0·175	0·200	0·350	0·325	0·225	0·231	0·222
1908–9	0·400	0·221	0·375	0·175	0·219	0·525	0·142	0·158	0·263	0·175	0·142	0·263	0·211
1909–10	0·294	0·092	0·200	0·125	0·133	0·075	0·117	0·300	0·275	0·250	0·263	0·238	0·179
1910–11	0·600	0·325	0·175	0·200	0·281	0·183	0·250	0·450	0·375	0·142	0·313	0·450	0·272
1911–12	0·375	0·213	0·163	0·167	0·275	0·200	0·108	0·500	0·375	0·142	0·250	0·117	0·190
1912–13	0·139	0·213	0·142	0·117	0·183	0·850	0·250	0·300	0·175	0·263	0·525	0·350	0·239
1913–14	0·325	1·063	0·250	0·275	0·400	0·125	0·092	0·150	0·550	0·288	0·225	0·300	0·323
1914–15	0·350	0·450	0·213	0·100	0·142	0·175	0·283	0·263	0·200	0·375	0·238	0·213	0·211
1915–16	0·158	0·400	0·213	0·100	0·200	0·158	0·200	0·150	0·225	0·225	0·225	0·575	0·232
Averages:													
1905–1916	0·292	0·307	0·194	0·144	0·190	0·180	0·166	0·262	0·297	0·218	0·250	0·264	0·225
1888–1916	0·244	0·220	0·186	0·167	0·179	0·193	0·180	0·245	0·253	0·199	0·204	0·205	0·203

TABLE 6. *Average monthly amount of chlorine in rain falling at Rothamsted.*

Sept. 1, 1877 to Aug. 31, 1916	Rainfall Average 39 years Inches	Chlorine Per million	Chlorine Per acre (lb.)
September ...	2·15	1·87	0·91
October	3·24	2·56	1·88
November ...	2·82	2·86	1·82
December ...	2·89	3·27	2·14
January ...	2·14	3·75	1·82
February ...	2·10	3·63	1·72
March	2 16	3·43	1·68
April	1·82	2·50	1·03
May	2·10	1·93	0·92
June	2·55	1·29	0·74
July	2·47	1·09	0·61
August	2·78	1·29	0·81
April–September	13·86	1·60	5·02
October–March ...	15·35	3·18	11·06
Whole year ...	29·21	2·43	16·08

TABLE 7. *Chlorine in rainwater collected at Rothamsted in parts per million.*

(For previous results see this *Journal*, 1905, 1, 303.)

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July	August	Year
1905–6	2·96	3·00	3·12	3·36	2·04	3·48	4·35	5·75	3·45	1·05	3·71	1·85	2·78
1906–7	3·98	1·65	2·28	3·20	6·72	3·35	4·68	2·21	2·51	2·25	1·56	1·05	2·55
1907–8	2·85	2·93	3·00	3·00	7·13	6·24	2·70	2·71	1·88	1·14	1·32	2·43	2·91
1908–9	2·70	1·95	6·93	4·05	5·93	10·27	2·25	1·91	2·90	1·22	0·98	1·29	2·41
1909–10	2·88	3·12	6·60	2·16	3·60	4·23	2·25	4·22	2·34	1·05	1·82	1·29	2·76
1910–11	3·56	4·65	2·33	4·08	6·63	4·41	3·60	3·39	2·40	1·89	1·61	2·43	3·39
1911–12	1·97	2·67	3·11	2·22	2·60	2·91	2·72	6·42	2·28	1·14	1·38	1·08	2·13
1912–13	2·18	2·90	2·60	2·70	3·33	8·25	4·26	2·67	1·56	1·83	1·37	1·77	2·85
1913–14	2·75	2·22	2·07	6·06	6·82	3·50	2·19	2·91	3·57	1·56	1·29	1·41	2·50
1914–15	3·08	3·12	3·45	3·18	2·25	3·66	3·03	2·86	1·34	1·73	1·31	0·87	2·56
1915–16	1·17	2·94	3·57	3·69	4·88	3·68	4·91	2·82	1·77	1·59	0·98	1·40	3·03
Average for period 1905–1916	2·56	2·91	3·06	3·19	3·79	4·09	3·29	2·87	2·12	1·44	1·37	1·43	2·73
Average for whole period 1877–1916	1·87	2·56	2·86	3·27	3·75	3·63	3·43	2·50	1·93	1·29	1·09	1·29	2·43

TABLE 8. *Chlorine in rainwater collected at Rothamsted.*

Harvest year Sept. 1 to Aug. 31	Rainfall in inches	Parts per million	Lbs. per acre
1901–2	23·26	2·81	14·81
1902–3	31·26	2·52	17·84
1903–4	31·50	2·76	19·66
1904–5	25·31	2·66	15·24
1905–6	23·77	2·78	14·94
1906–7	29·37	2·55	16·95
1907–8	30·12	2·91	19·86
1908–9	24·94	2·41	13·62
1909–10	30·93	2·76	19·33
1910–11	*26·77	3·39	*20·55
1911–12	39·88	2·13	19·23
1912–13	27·32	2·85	17·61
1913–14	25·01	2·50	14·17
1914–15	37·87	2·56	21·90
1915–16	35·62	3·03	†24·40
Average for period 1901–1916	29·53	2·70	18·01
Av. for whole period 1877–1916	29·21	2·43	16·08

* 11 months only: April figures not included.

† August value estimated.

TABLE 9. *Nitrogen as ammonia and nitric acid and chlorine in rainwater collected at Rothamsted in seven periods of four years, Sept.* 1, 1888 *to Aug.* 31, 1916.

September 1 to August 31	Rainfall in inches	NITROGEN Lbs. per acre As NH_3	NITROGEN Lbs. per acre As N_2O_5	NITROGEN Lbs. per acre Total	NITROGEN As NH_3	NITROGEN As N_2O_5	CHLORINE Lbs. per acre
1888–9 to 1891–2	27·65	2·815	0·981	3·796	74·2	25·8	12·25
1892–3 to 1895–6	27·95	2·633	1·081	3·714	70·9	29·1	14·35
1896–7 to 1899–1900	28·12	2·682	1·264	3·946	68·0	32·0	17·90
1900–1 to 1903–4	27·58	2·982	1·435	4·417	67·5	32·5	17·23
1904–5 to 1907–8	27·14	2·542	1·276	3·818	66·6	33·4	16·75
1908–9 to 1911–12	31·03	2·974	1·477	4·451	66·8	33·2	18·48
1912–13 to 1915–16	31·46	1·837	1·747	3·584	51·3	48·7	19·54

(*Received April* 15*th*, 1919.)

THE EFFECT OF POTASSIUM SALTS ON THE ANATOMY OF *DACTYLIS GLOMERATA*[1].

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PART I.

POTASSIUM compounds are known to play an important rôle in the metabolism and growth of plants. They are shown to be essential plant nutrients by water culture experiments; without these compounds plants are reduced in size, have an unhealthy colour, and eventually die. Potassium is found abundantly in embryonic tissues, and may possibly be concerned with the construction of protein material.

It is well known that potassium salts, unlike those of sodium, profoundly affect the processes of assimilation and translocation. Carbohydrate formation practically ceases in the absence of potassium salts, and where translocation or storage is taking place, an increased percentage of potassium is found. The amount of root formation in starchy or sugar-storing root crops is reduced by decreasing the amount of potassium, even while the leaf area, and therefore the area available for photosynthesis, remains normal. Thus, in the Rothamsted mangold plots, the yields per acre are:

	Leaf lbs. per acre	Root lbs. per acre	Root per lb. leaf weight
Potassic fertiliser supplied	8508 lbs.	40,128 lbs.	4·7 lbs.
No potassic fertiliser supplied	7255 „	14,684 „	2·02 „

Where potassium salts are omitted, there is a small decrease in the weight of leaf produced, but the amount of root in the same crop is reduced to less than half.

Potassium plays an important part in the synthesis of protein, and is found in large quantities in rapidly growing parts of plants (1, 2). Loew (2) has put forward the suggestion that this property of potassium salts is due to their condensing action on organic substances.

[1] Thesis approved for the degree of Master of Science in the University of London.

The resistance of the plant to disease is very dependent on the supply of potassium salts; fungoid and other pests find potash-starved plants an easy prey, while neighbouring plants with a better potassium supply remain much more healthy, though exposed to the same infection.

In addition to these effects, there are others which, though equally well marked, have received less attention from investigators. One of the most striking is the effect on the strength of the stem. Though growth is promoted in a wet season, there is considerable weakening of the stem, and, in heavy winds, a consequent tendency for the plant to fall down under its own weight. The application of manures, especially of those rich in nitrogen, increases this tendency, and since harvesting of "lodged" crops is extremely difficult, the amount of growth-producing manures which can safely be applied is limited by the ability of the straw to stand up against rain and wind. It has, however, been demonstrated in the field experiments at Rothamsted that an application of potassium salts strengthens the straw in some way, so that larger quantities of growth-producing fertilisers can be applied. This strengthening has been observed elsewhere. R. P. Wright (3) in 1896 found that an application of kainit ($MgSO_4 . KCl . 3H_2O$) which contains 12·5 per cent. K_2O, increased the solidity rather than the length of straw in oats. Lodging occurred where sodium nitrate was applied alone, but where kainit was also added there was no lodging, and the crop stood well. Close, White and Ballard (4) state that whereas carnations produce weak stems with nitrogenous fertilisers, they were strengthened by phosphates, and to a smaller extent by potassium salts. This strengthening is also the general experience of farmers who use potassic fertilisers.

This might be due to strengthening of the mechanical tissues, or to some other effect, such as an influence on the chemical composition of the walls or on the physiological condition of the plant.

Few attempts have been made to account for this effect of potassium salts, and they have led to no conclusive results.

Copeland (5) found that potassium salts increased the turgor of root cells to a greater extent than do sodium salts, and that they are stored in considerable quantities in the cell sap. If this were the case throughout the plant the increased turgor would considerably enhance the standing power of the plant. This question of turgor has not been fully investigated and may prove of great importance in relation to the subject under consideration.

Kissel (6) found that potash had no definite effect on the stems of oats, but in the case of grasses, especially of *Avena elatior*, the presence

of potash was accompanied by a reduction in the thickness of the walls, and an increase in the diameter of the lumen. Vogeler and Thiele (7) obtained inconclusive results.

Dassonville (8) investigated the effect of mineral salts on many plants, using both water cultures and pot-cultures in his experiments. He grew plants in culture solutions containing potassium chloride side by side with those in which this salt had been replaced by sodium chloride. With potassium chloride the plants grew much more vigorously than with the sodium salt, but in the case of grasses and cereals, the lowest internode was so weak that the plant fell, while those with sodium chloride were still standing. This he attributed to a weakening effect of potassium salts. Sections revealed more intense lignification in plants grown without potassium chloride. This result is clearly not in accordance with the facts observed in the field, and cannot, therefore, be accepted as final. Dassonville himself found that the stunted plants grown in distilled water showed the most intense lignification of all.

This last result of Dassonville suggests that starvation promotes rapid lignification in the young stages, possibly because reserves of carbohydrates in the seed go to form cell-wall material in the absence of minerals necessary for the formation of proteins. It may be then that the thickening of plants in the sodium chloride culture solution is due to starvation rather than to the effect of sodium itself. The unusual weakness of the plants which were supplied with potash was presumably due to the unsuitability of the solution Dassonville used for cereals; barley grown in the stronger solution used at Rothamsted has never been known to collapse, and can be brought to fruit, while specimens of *Lolium perenne* have been growing in the Rothamsted solution for more than eight months, and many of them are still perfectly healthy.

It may be that the effect of potassium salts varies with the period of growth—that one result would be obtained early in the life of the plant, but a totally different one later. Dassonville examined his plants at one stage only—the time was determined by the collapse of his plants.

During the summer of 1917 the effect of potassium salts on the anatomy of Dactylis was investigated, and the results are set out in this paper. Plants were collected both from field plots which had received potassium fertilisers, and from those which had not. The potassium salts in the soil were sufficient to prevent complete potash starvation where no such salts had been added, so that one difficulty of Dassonville's experiments was removed. Moreover, material was collected at short intervals during the summer, from the time when flowering stems were

just appearing, until a few days before the hay was cut, so that all stages of growth were investigated.

A large number of measurements of cell-walls and lumina were taken at these various stages; but they do not show that potassium has a well-marked effect.

In the presence of added potassium salts, especially in the early stages of growth, the thickening of the sclerenchyma cell-walls was less pronounced, and not more so, as would be expected if changed wall thickness were the cause of the additional strength. As the summer progressed, the difference became smaller, and in the case of plants which had in addition received nitrogenous manures, the cell-walls were as thick as those of plants receiving no potassic fertiliser.

The addition of potash, however, caused considerable increase in the rate of growth, and nearly doubled the crop. It is possible, therefore, that in the early stages material was used for growth that might otherwise have been used for wall thickening. The circumstances that the walls ultimately had the same thickness as in plants which had not been supplied with potash shows that the rate of thickening was greater with added potassium salts, and may, therefore, indicate that these substances do actually help in thickening the walls.

Repetitions of the observations in other seasons, comparison being made with the rate of growth, would be necessary to decide this question. This possibility receives some support from the fact that on May 31st the plants which had been supplied with potash had the thicker walls (see Figs. 6 and 18).

In the xylem potassium salts had no effect on the wall thickness, and on the diameter of the lumen the effect was irregular and inconclusive.

Further evidence was obtained by studying the ratio of lumen to wall in the same tissues. The ratio of the lumen to the wall in each individual cell was taken as a numerical expression of its mechanical strength. While the material of the wall remains the same, a cell where the lumen is small in proportion to the thickness of the cell-wall would be mechanically stronger than one where it is large. It is not safe to lay too much stress on this, because it is possible that the cell-wall material would differ in plants which have received dissimilar foods. If, however, it were true that the mechanical effect of potassium salts were due to their influence on the anatomical character of the plant, then this ratio would be inversely proportional to the mechanical strength of the cells. The results lead to the conclusion that the strengthening effect of potas-

sium salts on the stems of grasses is not due to their influence on the structure of the mechanical tissues, and that potash has some other effect on the plant to which this strengthening must be attributed.

PART II. EXPERIMENTAL METHODS.

The plant selected for this investigation was *Dactylis glomerata*. This grass is a fairly common constituent of the hay from all the grass plots at Rothamsted from which material was collected[1], and it puts up a stout flowering stem from which suitable sections for investigation could easily be cut. Its stem structure is, moreover, typical of the Gramineae and it is among cereals and grasses that lodging takes place to a serious extent.

The material was collected from plots which had been receiving the following manurial treatments for nearly sixty years.

Plot	Manure	$(NH_4)_2SO_4$ lbs.	Super-phosp. cwts.	K_2SO_4 lbs.	Na_2SO_4 lbs.	$MgSO_4$ lbs.
7	Complete minerals	—	3·5	500	100	100
8	Minerals without potassium salts	—	3·5	—	250*	100
9	Complete minerals + ammonium salts	400	3·5	500	100	100
10	Minerals without potassium salts + ammonium salts	400	3·5	—	250*	100

* Reduced to 100 lbs. in 1905.

With this material it was possible to investigate the effect of potassium salts both in the presence and absence of nitrogenous manures.

The effect of potash manures in preventing lodging is well exemplified on the grass plots. Frequently the grass on plots without potash, such as 8 and 10, was completely flattened, while that on the neighbouring plots 7 and 9 was still standing.

If, then, this effect is actually due to the influence of potash on the anatomy, plants from these plots should show marked anatomical differences.

The summer of 1917, when this investigation was carried out, was remarkably dry during the growing period of the hay, and it was not until a few days before it was cut that heavy rains caused a certain amount of lodging to take place.

[1] Attempts were made to procure material from plants grown in water cultures, but under these conditions no flowering stem was formed.

The rainfall during the period of investigation is shown in Fig. 1. Heavy rains occurred at the beginning of the investigation, but from May 25th onwards rainfall was scanty until after the last material had been collected.

The spring of 1917 was very cold, but during the period of investigation the shade temperature was on the whole high, as may be seen on an examination of Fig. 2, in which the maximum and minimum shade temperatures on each day are plotted.

The crop weight for the summer was not widely different from the average for the ten years 1906–1915, but the effect of potash on the yield was intensified, possibly by the dryness of the year.

Yield per acre, 1st crop only.

	Plot 7 Potash added	Plot 8 Potash omitted	Plot 9 Potash added	Plot 10 Potash omitted
1918	33·2 cwt.	17·3 cwt.	33·6 cwt.	18·3 cwt.
Av. '06–'15	30·0 „	19·3 „	36·8 „	25·6 „

Dactylis was not plentiful on any of the plots, and very little occurred on Plot 10. Rumex indicating the acidity of the soil was plentiful on the two plots (9 and 10) which had received ammonium sulphate.

Specimens of the third internode below the inflorescence were collected at intervals during the summer of 1917, on May 16th, May 31st, June 8th, June 15th and June 26th. On May 16th the inflorescence could be felt as a swelling hidden by the younger leaves, while the stem was but slightly developed and not more than from two to eight centimetres long. On this occasion, the first internode below the inflorescence was used, and five specimens taken from each plot. On May 31st the inflorescences were above the leaves, and the stem well developed; ten specimens were taken from each plot. On subsequent dates only five specimens were taken, on account of the small amount of Dactylis occurring where nitrogenous manures had been applied. The hay was cut on July 6th.

The stems were fixed for twenty-four hours in acetic alcohol, and washed and preserved in 70 per cent. spirit. A section of each specimen was cut, stained with safranin and aniline blue, and mounted in Canada balsam. Measurements were made by means of an eye-piece micrometer, using a Zeiss $\frac{1}{12}$ inch oil immersion objective.

The internodes selected were enclosed by the leaf-sheath, and were slightly flattened and winged (Fig. 3). An outer ring of bundles was embedded in the sclerenchymatous zone, which adjoined the epidermis

just outside the bundle, but was separated from it at other points by thin plates of assimilatory tissue.

Within the sclerenchymatous zone is a large-celled ground tissue, through which the larger bundles run. The sharpness of the division between sclerenchyma and cortex varies in the different specimens. The centre of the stem is occupied by a large lysigenic cavity which extends throughout the length of each internode.

The bundles are collateral, and are surrounded by a badly defined sheath, whose cells are slightly lignified; the xylem consists as a rule of one or occasionally two protoxylem elements, two large metaxylem vessels and a few smaller elements. As no significant difference was observed in the general appearance of the sections obtained from different plots, more detailed measurements of cells were made to find if any finer difference existed. Attention was concentrated on the cells of the sclerenchymatous zone, whose function is almost entirely mechanical, and of the large metaxylem elements, which though not to any extent mechanical in function, have become lignified, and provide material which might throw light on the problem in hand. Measurements were made of the thickness of the cell-walls, and the diameter of the lumina, while the ratio of lumen to wall was calculated for each cell.

In most cases ten cells or vessels from each slide were measured, but occasionally where the material was insufficient, twenty measurements were made of some of the sections. In either case, the total number of readings was eighty for the xylem, and fifty for the sclerenchyma, and these readings were taken from a number of individual plants varying from four to eight. A few sections showed marked differences from others from the same plot, and were rejected as anomalous.

The readings thus taken showed considerable variation among themselves; in one case, for instance, the width of the lumen in sclerenchymatous cells ranged from 4μ—16μ (Plot 7, June 26th), slide *e*. This variation is unavoidable, but the validity of the result is considerably enhanced by the large number of readings taken, and where the "probable error" has been calculated, the significance of a difference between two means can be decided; a significant difference is one that is considerably greater than the sum of the "probable error" of the two means. Thus, the means of the fifty readings for the diameter of the lumina of sclerenchyma from stems of Plot 7 material (where full minerals, including potash, were applied) are as follows:

Date	May 16th	May 31st	June 8th	June 15th	June 26th
Mean	15·6 ± ·27μ	8·04 ± ·23μ	10·1 ± ·32μ	7·5 ± ·21μ	7·8 ± ·22μ
% error	1·73 %	2·86 %	3·16 %	2·8 %	2·83 %
Difference of means	7·56	2·06	2·60	·30	
Sum of "errors"	·50	·55	·53	·43	

The table shows that in this typical case, the deviation from the means does not exceed 5 per cent. and that it is possible to obtain significant differences between the means on different dates, and, as will be seen later, in material from different plots. In this case the differences between successive measurements were significant until June 15th, but between June 15th and June 26th they were too small to be considered.

RESULTS.

The sclerenchymatous zone consists of rather small isodiametric cells, with heavily lignified walls. In all stages examined the living protoplast was actively secreting cell-wall material, and the walls were therefore becoming thickened as the season progressed.

The xylem vessels on the other hand were no longer living cells; it is, therefore, impossible that new wall material should be deposited.

DEVELOPMENT UNDER NORMAL CONDITIONS OF POTASH SUPPLY.
(Plot 7, full minerals supplied.)

A. *In sclerenchyma.*

The rate of change, both in wall thickness and in the diameter of the lumen, is by no means regular. On May 16th, when the material was first collected, the mean wall thickness was 1·88μ. The stems at this date were very immature, and not more than two or three centimetres long. The degree of differentiation varied considerably between individuals at this early stage; two or three days start was enough to allow lignification to be well ahead in some specimens. In addition, as the sections did not take the stains readily, accurate measurement under the high power used was extremely difficult. In consequence, less reliance can be placed on this set of measurements than on those of a later date, but they are sufficiently different from those of the succeeding set to show that a considerable change has taken place.

Plot 7. *Sclerenchyma.* Complete mineral manures.

Date	May 16th	May 31st	June 8th	June 15th	June 26th
Walls	1·88 ± ·09μ	2·81 ± ·11μ	2·39 ± ·06μ	5·06 ± ·10μ	4·27 ± ·13μ
Lumina	15·6 ± ·27μ	8·1 ± ·23μ	10·1 ± ·32μ	7·5 ± ·21μ	7·8 ± ·22μ
Ratio	9·37	3·22	4·3	1·57	2·12

The most striking feature in the progress of wall thickness in the material supplied with full minerals (Fig. 6) is the sudden increase between June 8th and June 15th. During the periods both preceding and following this increase the thickness diminished by an amount which, though slight, seems to be outside the limit of variation.

The corresponding changes in the diameter of the lumen are shown in Fig. 7. On May 15th the cells were large and little differentiated, but by May 31st the mean diameter had decreased by $7{\cdot}5\mu$. During the following week (June 1st to June 8th) an expansion of the cells occurred —the lumen increased while the wall thickness diminished. This increase in the lumen was not observed in any other material except that from Plot 10, where it will be remembered no potash was supplied although ammonium salts were given as a source of nitrogen. It does not, therefore, appear to be the result of supplying potassium salts to the plant.

As already explained, the ratio of wall thickness to lumen may be taken as a measure of mechanical strength of the individual cell. For purposes of convenience the ratio has been inverted. Fig. 8 shows the progress of the ratio of lumen to wall in Plot 7 material which followed closely that of the lumen diameter. The course of the curve indicated that the mechanical strength of the cells increased considerably during the season but that the increase was not uniform throughout, and on one occasion, between June 1st and June 8th, a decrease occurred. Reference to the weather curves shows that during this week there was moderate rainfall after a short period of drought. This might account for the stretching of the cells, but does not explain the fact that the same thing is not seen in material from the other plots. The thickening and recoveıy from this weakening influence during the ensuing week must be emphasised.

B. *In metaxylem vessels.*

Plot 7. *Xylem.* Complete mineral manures.

Date	May 16th	May 31st	June 8th	June 15th	June 26th
Walls	$1{\cdot}92\mu$	$2{\cdot}16\mu$	$1{\cdot}93\mu$	$2{\cdot}04\mu$	$2{\cdot}16\mu$
Lumina	$24{\cdot}6\mu$	$26{\cdot}9\mu$	$29{\cdot}6\mu$	$31{\cdot}45\mu$	$27{\cdot}8\mu$
Ratio	93·41	14·44	16·19	15·56	13·38

As may be seen from the curve in Fig. 9 there was very little change in the wall thickness in the material receiving potash throughout the summer. The slight apparent drop between June 1st and June 8th was possibly due to experimental error, but is too great to be dismissed as such. It occurred during the same period as the similar decrease in the sclerenchyma walls.

In the diameter of the lumen (Fig. 10), a steady increase occurred from May 16th until June 15th. After this it decreased slightly until the end of the investigation. The change during the first week in June was again similar to that seen in the sclerenchyma, but cannot here be attributed to increased turgor.

The ratio, like the mean lumen diameter, increased until June 8th, showing in the early stages a gradual loss of mechanical strength in the individual cells. From that date onwards, however, it diminished, and finally on June 26th reached its original value of 13·4μ (Fig. 11).

These changes in the dimensions of the xylem vessels cannot be connected with wall deposition or turgor changes, such as occur in the living cells of the sclerenchyma.

COMPARISON OF DEVELOPMENT IN PRESENCE AND ABSENCE OF POTASSIUM SALTS WHERE NO NITROGEN FERTILISER HAS BEEN APPLIED.

Plot 8 is supplied with a yearly dressing similar to that given to Plot 7 except that potassium salts are omitted; differences between plants from the two plots can therefore be attributed to the influence of potassium salts.

A. *Sclerenchyma*.

Plot 8. Minerals without potassium salts.

Date	May 16th	May 31st	June 8th	June 15th	June 26th
Walls	3·3 $\pm$·09μ	2·07$\pm$·02μ	3·04$\pm$·07μ	5·3 $\pm$·12μ	5·44$\pm$·09μ
Lumina	15·1 $\pm$·29μ	9·4 $\pm$·26μ	9·3 $\pm$·22μ	5·6 $\pm$·12μ	6·6 $\pm$·22μ
Ratio	3·51	4·60	3·21	1·12	1·24

Plot 7. Complete minerals.

Walls	1·88$\pm$·09μ	2·81$\pm$·11μ	2·39$\pm$·06μ	5·06$\pm$·10μ	4·27$\pm$·13μ
Lumina	15·6 $\pm$·27μ	8·1 $\pm$·23μ	10·1 $\pm$·32μ	7·5 $\pm$·21μ	7·8 $\pm$·22μ
Ratio	9·37	3·22	4·3	1·57	2·12

In the case of the earliest material collected, the stem sections of plants from Plots 7 and 8 showed a striking difference in the structure of their mechanical tissue. Those from Plot 8, where no potash manure was added, possessed a well-marked sclerenchymatous zone, whose cell-walls readily took a basic stain, and which were twice and sometimes three times as thick as those in Plot 7 stems, which had received potassium salts. In the latter the sclerenchyma was hardly differentiated; it could only be distinguished from ground tissue by the characteristic refractive properties of its walls and would not take such stains as aniline safranin (Figs. 4 and 5).

In each set, however, one anomalous plant occurred. One section from Plot 7 showed well lignified sclerenchyma, while one from Plot 8 was hardly differentiated. These plants had been influenced by some other factor and for this reason their measurements were omitted from the mean values.

The wall thickness in Plot 8 material is shown in Fig. 6.

Between May 16th and May 31st it decreased by $1{\cdot}2\mu$, while during the same period an increase was observed in the case of plants which had received potassium manures. It is difficult to account for this fall, which cannot be due to stretching, for at the same time the lumen has decreased. From this date onwards the thickness continued to increase, until June 15th, when maximum development was attained. The wall thickness throughout, with one exception (on May 31st), was greater than in Plot 7 material; this difference was most marked in the very young stages.

Reference to Fig. 7 shows that the lumina in absence of potassium salts were smaller than where these were applied, with the exception of those in stems obtained on May 31st. The general tendency to additional strength of the cells was shown here by Plot 8 material in the smaller diameter of the lumen as well as in the greater thickness of the walls, and the reversal of this general tendency on May 31st was again exemplified.

Throughout the whole period there was a slow decrease in the lumen, except during the last ten days when a slight increase occurred, and during the first week in June when there was no change.

In Plot 8 material an increase in the ratio of lumen to wall occurred during the first period (May 16th—May 31st) due both to the rise in wall thickness and the decrease of the mean lumen diameter (Fig. 8). After this date the ratio fell slowly till June 15th, and thereafter remained constant till June 20th.

The ratio was below that found for Plot 7 material, except on May 31st. From June 8th onwards, however, the difference was slight. This comparison indicates that where potassium salts have been added (Plot 7) the mechanical strength, as measured by the reciprocal of the ratio of lumen to wall, was reduced, especially in the early stages of growth. On May 31st, however, this conclusion does not hold; for some reason the plants treated with potash had thicker walls and a smaller lumen than where it had been omitted.

The wall thickness in Plot 8 as shown in Fig. 9 again remained fairly steady throughout the season; from May 31st onwards a very slight

increase was seen which, however, was too small to be considered significant. There was no significant difference between the plants from the two plots in the thickness of xylem walls.

B. *In xylem.*

Plot 8. Receiving minerals without potassium salts.

Date	May 16th	May 31st	June 8th	June 15th	June 26th
Walls	1·96μ	1·87μ	2·02μ	2·08μ	2·24μ
Lumina	24·8μ	30·4μ	35·3μ	27·0μ	31·3μ
Ratio	12·50	16·85	18·22	13·20	14·18

Plot 7. Full minerals.

Walls	1·92μ	2·16μ	1·93μ	2·04μ	2·16μ
Lumina	24·6μ	26·9μ	29·6μ	31·45μ	27·8μ
Ratio	13·4	14·44	16·19	15·56	13·38

At first the mean lumina were practically equal, but afterwards, from May 31st the diameters were considerably higher where potassium salts had been withheld, except on June 15th. The sudden drop between June 8th and June 15th may be due to extreme pressure exerted by rapidly expanding cells of the ground tissue (Fig. 10).

Fig. 11 shows that the ratios of lumen to wall were equal at the beginning of the investigation, but on May 31st and on June 8th, they were higher where no potassium salts had been applied. On June 15th the ratio in Plot 8 material had fallen considerably below that in plants which had received potash but by June 26th had risen slightly above it.

In the xylem the addition of potassium salts had no effect on the wall thickness, but reduced the diameter of the lumen. This is the reverse of the result obtained from sclerenchyma, but it must be remembered that the two types of cell are not comparable. The xylem attained a permanent and fixed condition before the earliest material was collected, and changes during the period of observation may be attributed to purely mechanical forces acting on the vessels. In the sclerenchyma the weakening effect of potash was most marked in the early stages, and, had it been possible to collect material later in the season this discrepancy might have been found greatly reduced.

EFFECT OF POTASSIUM SALTS WHEN NITROGEN FERTILISERS ARE ADDED.

The effect on crops of the addition of nitrogen fertilisers is to increase the luxuriance of the vegetation, frequently at the expense of mechanical strength. Microscopic investigation has revealed the fact that nitrogen

decreases the thickness of walls and increases the lumen (9). Before the effect of potash in the presence of nitrogenous manures can be properly investigated, it is necessary to observe the effect of these manures themselves. For this purpose material from Plot 7, receiving full minerals, was compared with that from Plot 9, where full minerals and ammonium sulphate had been applied. This addition produces an alteration in two factors governing growth; firstly the amount of nitrogen is greater, and secondly the acidity of the soil is increased by the liberation of SO_4 ions. Possibly this acidity may account for the small amount of Dactylis to be found on Plots 9 and 10.

Comparison of Plots 7 and 9.

A. *Sclerenchyma.*

Plot 9. *Sclerenchyma*. Full minerals + ammonium sulphate.

Date	May 16th	May 31st	June 8th	June 15th	June 26th
Walls	1·16 ± ·03μ	2·13 ± ·05μ	2·8 ± ·07μ	4·64 ± ·15μ	4·8 ± ·10μ
Lumina	14·3 ± ·28μ	9·1 ± ·18μ	9·4 ± ·22μ	7·1 ± ·18μ	7·2 ± ·22μ
Ratio	12·77	4·49	3·65	1·69	1·61

Plot 7. Full minerals only.

Walls	1·88 ± ·09μ	2·81 ± ·11μ	2·39 ± ·06μ	5·06 ± ·10μ	4·27 ± ·13μ
Lumina	15·6 ± ·27μ	8·1 ± ·23μ	10·1 ± ·32μ	7·5 ± ·21μ	7·8 ± ·22μ
Ratio	9·37	3·22	4·30	1·57	2·12

The walls of plants grown on Plot 9 (Fig. 12) which received nitrogenous manure were, on the whole, thinner than those from Plot 7, but this difference was not maintained throughout the period of investigation. On two occasions, after the unexpected falls in the mean for Plot 7, the thickness was greater where nitrogen was applied. Plot 9 material gave a steady increase from beginning to end.

Contrary to expectation, the addition of nitrogen fertiliser did not increase the lumen, indeed, if anything it decreased it. From Fig. 13 it is clear that the mean for Plot 9 material, which has received ammonium salts, was slightly below that for Plot 7 on every date but May 31st, but the difference throughout was hardly significant and the course of the two curves was similar.

At first as can be seen from Fig. 14 the ratio of lumen to wall was higher in Plot 9 material than in that from Plot 7, but as the season progressed the difference became less marked until finally the means were practically equal.

The effect of nitrogen was not well marked. The cell-walls were thinner in the early stages only, but the diameter of the lumen was not

affected. The mechanical strength of the cell was, in consequence, reduced during the early stages, but later no difference was apparent. It is possible that the dryness of the season may account for the absence of nitrogen effect in the later stages. During May when the rainfall was greater, a weakening on the nitrogen plots was observed.

B. *In xylem.*

Plot 9. Full minerals + ammonium sulphate.

Date	May 31st	June 8th	June 15th	June 26th
Walls	1·87μ	2·27μ	2 16μ	2·08μ
Lumina	36·3μ	32 6μ	28·35μ	35·7μ
Ratio	20·49	14·83	13·62	17·49

Plot 7. Full minerals only.

Walls	2·16μ	1·93μ	2·04μ	2·16μ
Lumina	26·9μ	29·6μ	31·45μ	27·8μ
Ratio	14·44	16·19	15·56	13·38

In the material collected from Plot 9 on May 16th no measurements of the xylem were made as the vessels were crushed and distorted, and accurate observation was impossible.

The walls of the xylem appeared to be slightly thicker where nitrogen had been applied, except on May 31st (Fig. 15).

The lumen, however, was considerably greater where nitrogen had been added than with minerals only, except on June 15th. This difference was much more marked at the beginning and end of the period than on intermediate dates (Fig. 16).

The ratio of lumen to wall as seen in Fig. 17 indicates that where nitrogenous manure was added the mechanical strength of the vessels themselves was greater at the beginning and end of the period, but on the intermediate dates, June 8th and June 15th, was rather less than where minerals only were applied.

Comparison of Plot 9 with Plot 10.

A consideration of the effect of potassium salts in the presence of added nitrogen is now possible. Plot 10, like Plot 9, receives a dressing of ammonium sulphate and minerals, but here potassium salts are omitted.

From Fig. 18 it is clear that in the plots without added nitrogenous fertiliser the walls were thicker in Plot 10, where potash was withheld than in Plot 9 where it was supplied, except on one occasion (June 26th) when they were appreciably thinner, and on May 31st, when the means

were almost equal. The effect of potassium salts on the walls was very similar to that observed in the absence of nitrogen fertilisers; with these salts the walls are considerably thinner, in the early stages, but this difference is diminished as the season goes on.

A. *Sclerenchyma.*

Plot 10. Ammonium salts + minerals without potash.

Date	May 16th	May 31st	June 8th	June 15th	June 26th
Walls	$2{\cdot}63 \pm {\cdot}04\mu$	$2{\cdot}0 \pm {\cdot}03\mu$	$3{\cdot}59 \pm {\cdot}09\mu$	$5{\cdot}0 \pm {\cdot}61\mu$	$3{\cdot}94 \pm {\cdot}13\mu$
Lumina	$14{\cdot}5 \pm {\cdot}32\mu$	$9{\cdot}2 \pm {\cdot}18\mu$	$11{\cdot}1 \pm {\cdot}31\mu$	$6{\cdot}6 \pm {\cdot}16\mu$	$9{\cdot}1 \pm {\cdot}23\mu$
Ratio	4·86	4·68	3·36	1·34	2·77

Plot 9. Ammonium salts + full minerals.

Walls	$1{\cdot}16 \pm {\cdot}03\mu$	$2{\cdot}13 \pm {\cdot}05\mu$	$2{\cdot}8 \pm {\cdot}07\mu$	$4{\cdot}64 \pm {\cdot}15\mu$	$4{\cdot}8 \pm {\cdot}10\mu$
Lumina	$14{\cdot}3 \pm {\cdot}28\mu$	$9{\cdot}1 \pm {\cdot}18\mu$	$9{\cdot}4 \pm {\cdot}22\mu$	$7{\cdot}1 \pm {\cdot}18\mu$	$7{\cdot}2 \pm {\cdot}22\mu$
Ratio	12·77	4·49	3·65	1·69	1·61

During the first period the mean lumina were equal but after May 31st the means were slightly higher in the material without potash. During the first week in June there was a sudden increase in lumen in Plot 10 material similar to that observed in Plot 7 (full minerals), and a similar increase occurred between June 15th and June 26th (Fig. 19).

B. *Xylem.*

Plot 10. *Xylem.* Ammonium sulphate + minerals without potash.

Date	May 31st	June 8th	June 15th	June 26th
Walls	$2{\cdot}21\mu$	$2{\cdot}02\mu$	$2{\cdot}04\mu$	$2{\cdot}42\mu$
Lumina	$29{\cdot}4\mu$	$34{\cdot}7\mu$	$30{\cdot}9\mu$	$31{\cdot}7\mu$
Ratio	13·60	17·83	15·27	13·30

Plot 9. *Xylem.* Ammonium sulphate + full minerals.

Walls	$1{\cdot}87\mu$	$2{\cdot}27\mu$	$2{\cdot}16\mu$	$2{\cdot}08\mu$
Lumina	$36{\cdot}3\mu$	$32{\cdot}6\mu$	$28{\cdot}35\mu$	$35{\cdot}7\mu$
Ratio	20·49	14·83	13·62	17·49

On May 16th the ratio of lumen to wall in Plot 9 material was much higher than that in material from Plot 10 (Fig. 20), a result which is consistent with that obtained from Plots 7 and 8. On May 31st, and on subsequent dates, the readings for the two plots were almost equal; only on June 26th was the difference significant, and there the plants which had received potassium salts had the lower ratio. Again, on the view already expressed, the result obtained would imply that there is greater mechanical strength in the cells of the sclerenchyma where no potassium salts had been applied, but that the difference becomes less as the season advances. In the presence of nitrogen, by June 26th, the

greater rigidity was found in the material which had received potash, but this was not the case in Plots 7 and 8.

There was little difference between the wall thickness (shown in Fig. 21) in material from Plots 9 and 10; on May 31st and June 26th the thickness was greater without potassium salts, but on the two intermediate dates it was greater where they had been applied.

The lumen was considerably greater where potash was present on May 31st and June 26th, but on the two intermediate dates the plants which had been treated with potash had xylem vessels with smaller lumina (Fig. 22).

As the wall thickness was practically unaltered throughout, the ratio of lumen to wall, and therefore the mechanical strength of the cells (assuming as in other cases the similarity of wall material), was determined by the width of the lumina (Fig. 23).

In the case where nitrogen fertilisers were not applied the effect of potash on the xylem vessels is indefinite.

SUMMARY AND CONCLUSION.

Stems of *Dactylis glomerata* were collected from grass-plots which had received different manurial treatment as regards potash.

The yield of hay from these plots was in close agreement with the average which shows that the season during which the work was done was not abnormal.

The thickness of the wall, the diameter of the lumina and the ratio of the lumen to the wall were measured both in sclerenchyma and meta-xylem elements.

It was found that in the early stages the sclerenchyma walls were thinner where potash had been supplied, but that this effect was lost as the season progressed.

The lumina were larger in plants which had received potash, when nitrogenous fertilisers had not been added, but in the presence of ammonium salts, this effect was reversed.

In the xylem the thickness of the walls was unaltered whether potassic fertiliser were added or not. When no nitrogenous manures were added the diameter of the lumen was decreased in the presence of potash, but when ammonium salts had been applied, the diameter was increased by the application of potassic fertilisers.

The addition of potassium salts produced an increased ratio of lumen to wall but this effect gradually passed off. Presumably, therefore,

potassic fertilisers reduced the strength of mechanical cells in the early stages of growth. This conclusion however would not hold if potassium salts affected the composition of the wall.

From these results it is concluded that the rigidity of plants supplied with potassium salts is not the result of anatomical strengthening, but must be attributed to other causes, such as the influence of the salts on the physiological condition of the plant, or on its chemical composition.

This work was carried out partly in the Botanical Department of the Royal Holloway College, partly at Rothamsted Experimental Station, and I desire here to express my gratitude to Professor Benson and Dr E. J. Russell for their helpful criticism and advice during the course of the investigation.

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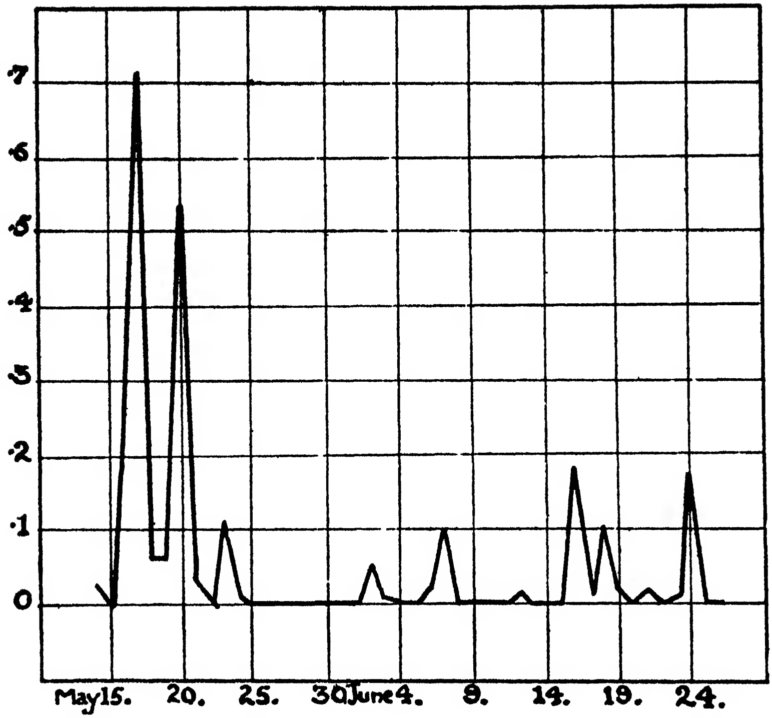

Fig. 1. Graph showing daily rainfall in inches during period of investigation.

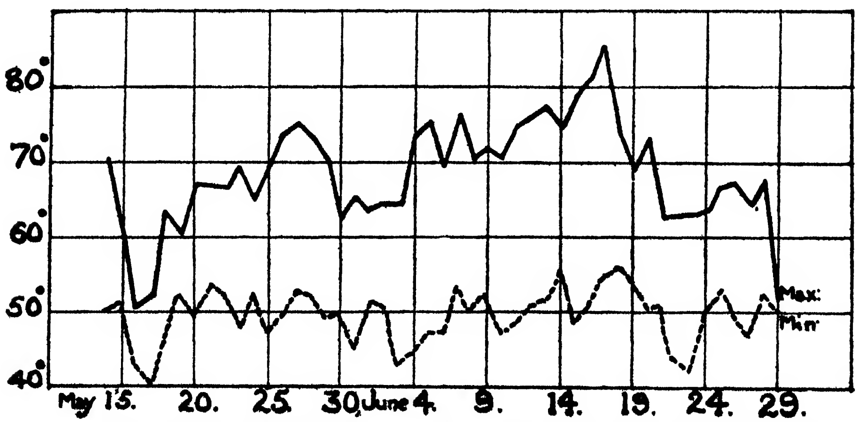

Fig. 2. Graph showing daily shade temperatures (maximum and minimum) during period.

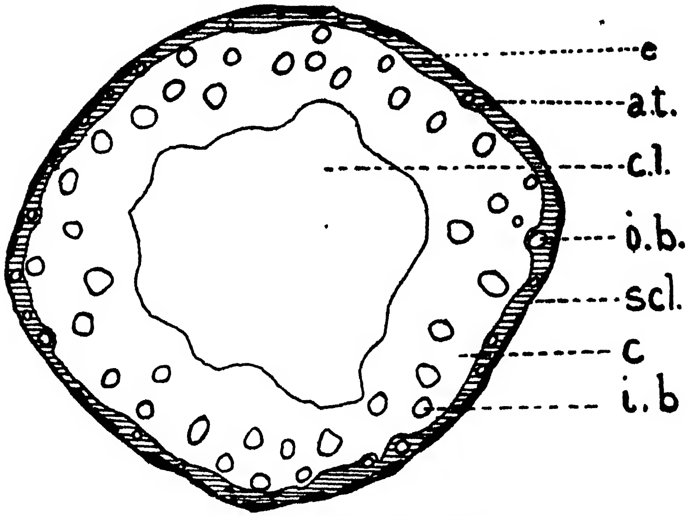

Fig. 3. Diagram of +-section of stem of *Dactylis glomerata.* *e* = epidermis, *a.t.* = assimilatory tissue, *c.l.* = central lacuna, *o.b.* = outer bundle, *scl.* = sclerenchyma, *c.* = cortex, *i.b.* = inner bundle.

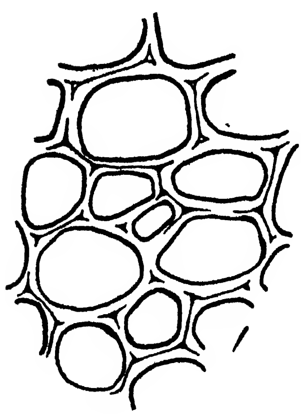

Fig. 4. Drawing of sclerenchymatous cells from young stem grown on plot with added potash.

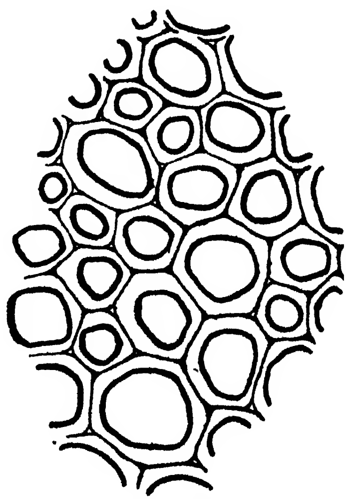

Fig. 5. Drawing of cells similar to those shown in Fig. 4, but from stem grown without added potash.

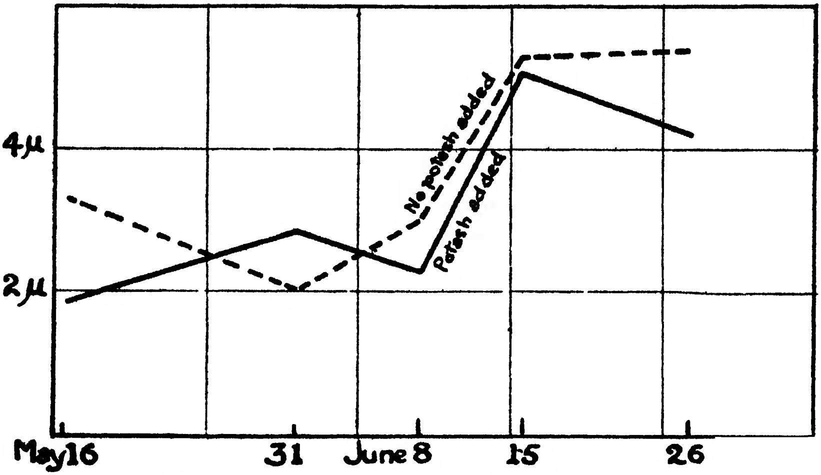

Fig. 6. Mean thickness of walls in sclerenchyma.

Full line —— Plot 7, with added potassium salts.
Broken line - - - Plot 8, without added potassium salts.

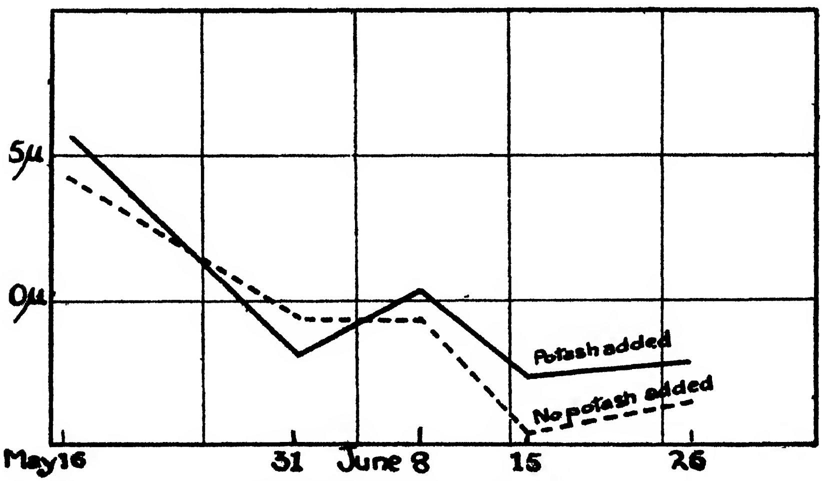

Fig. 7. Mean diameter of lumina in sclerenchyma.

Full line —— Plot 7, with added potassium salts.
Broken line - - - Plot 8, without added potassium salts.

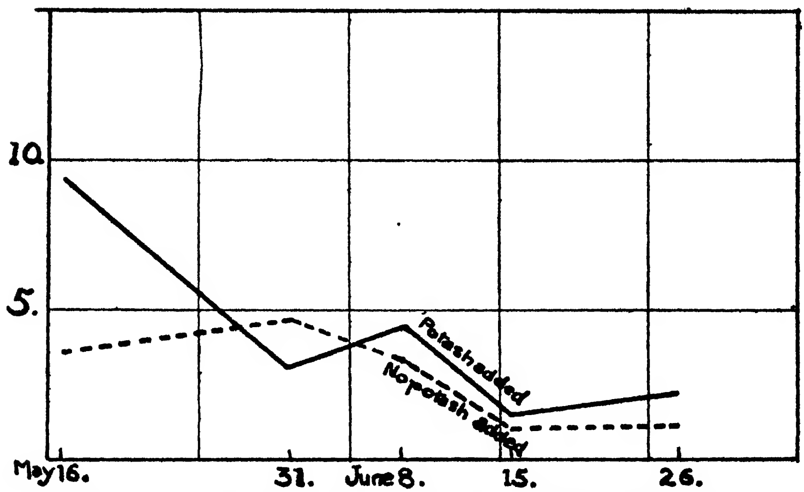

Fig. 8. Mean ratio of lumen diameter to wall thickness in sclerenchyma.

Full line —— Plot 7, with added potassium salts.
Broken line - - - Plot 8, without added potassium salts.

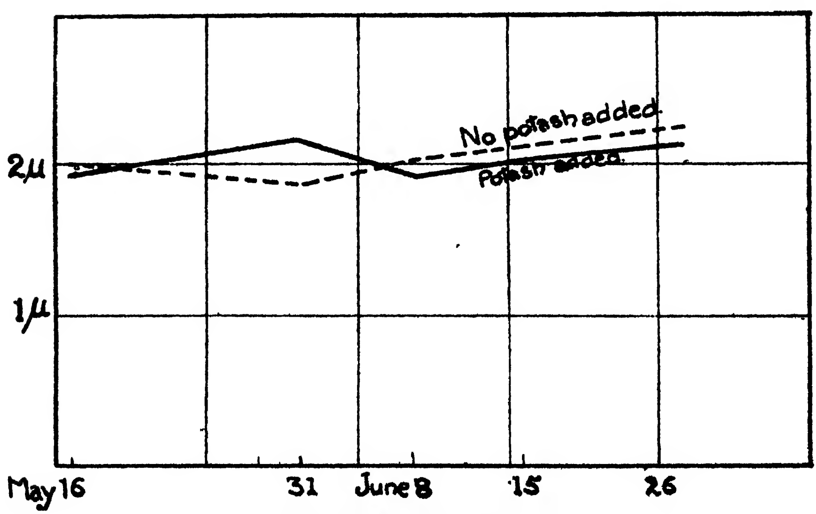

Fig. 9. Mean thickness of walls in xylem.

Full line —— Plot 7, with added potassium salts.
Broken line - - - Plot 8, without added potassium salts.

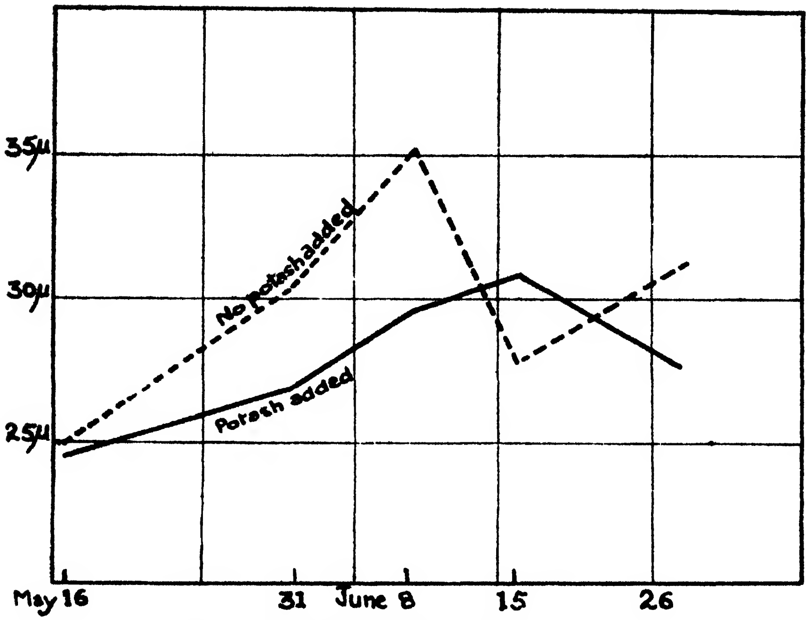

Fig. 10. Mean diameter of lumina in xylem.
Full line —— Plot 7, with added potassium salts.
Broken line - - - Plot 8, without added potassium salts.

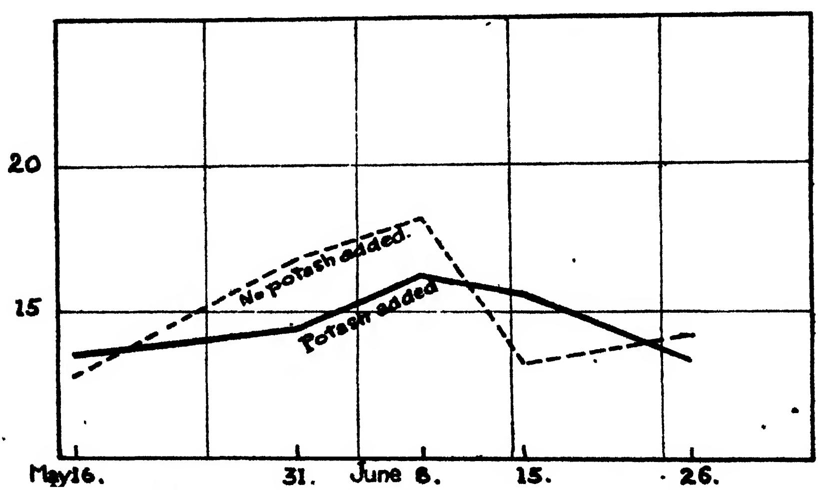

Fig. 11. Mean ratio of lumen diameter to wall thickness in sclerenchyma.
Full line —— Plot 7, with added potassium salts.
Broken line - - - Plot 8, without added potassium salts.

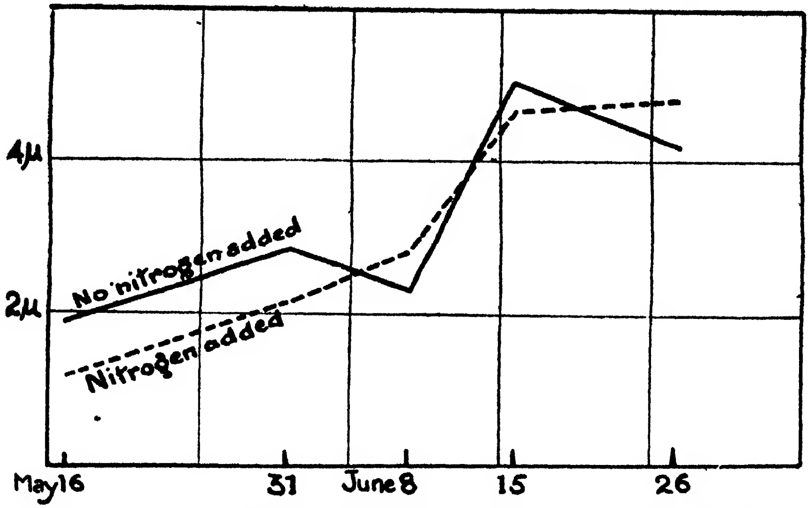

Fig. 12. Mean thickness of walls, sclerenchyma, in presence of added potash.
Full line —— Plot 7, no nitrogenous fertiliser added.
Broken line - - - Plot 9, nitrogenous fertiliser added.

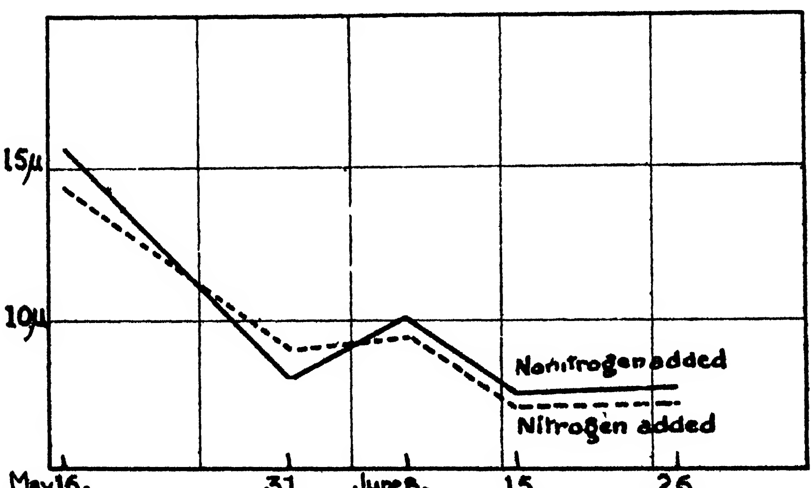

Fig. 13. Mean diameter of lumina, sclerenchyma, in presence of added potash.
Full line —— Plot 7, no nitrogenous fertiliser added.
Broken line - - - Plot 9, nitrogenous fertiliser added.

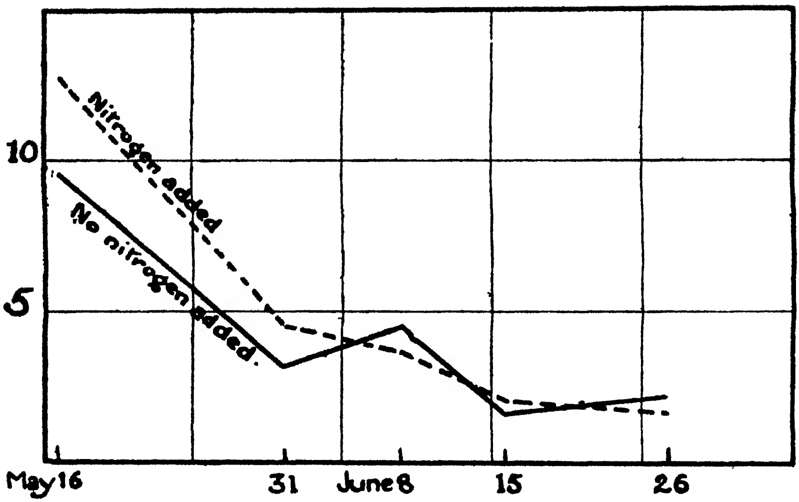

Fig. 14. Mean ratio of lumen diameter to wall thickness, in presence of added potash.

Full line —— Plot 7, no nitrogenous fertiliser added.
Broken line - - - Plot 9, nitrogenous fertiliser added.

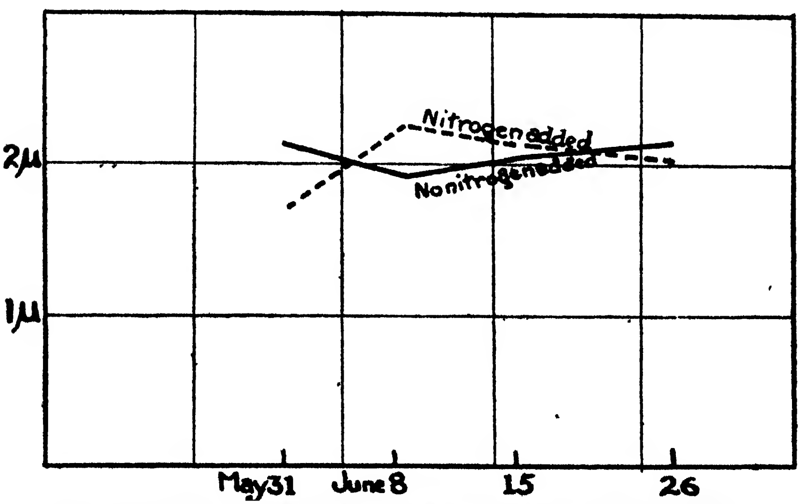

Fig. 15. Mean thickness of walls, xylem, in presence of added potash.

Full line —— Plot 7, no nitrogenous fertiliser added.
Broken line - - - Plot 9, nitrogenous fertiliser added.

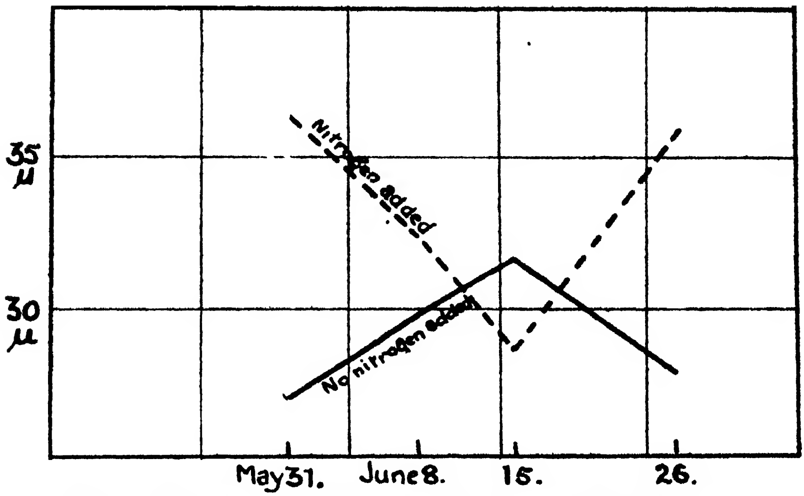

Fig. 16. Mean diameter of lumina, xylem, in presence of added potash

Full line —— Plot 7, no nitrogenous fertiliser added.
Broken line - - - Plot 9, nitrogenous fertiliser added.

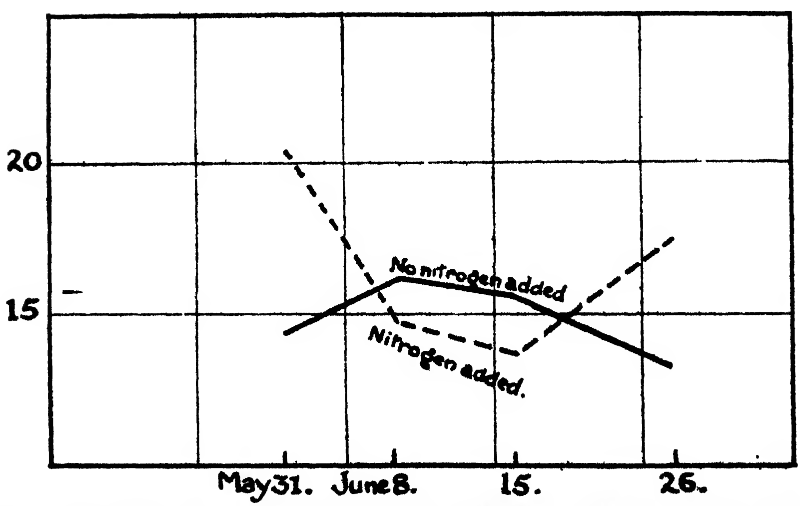

Fig. 17. Mean ratio of lumen diameter to wall thickness, in presence of added potash.

Full line —— Plot 7, no nitrogenous fertiliser added.
Broken line - - - Plot 9, nitrogenous fertiliser added.

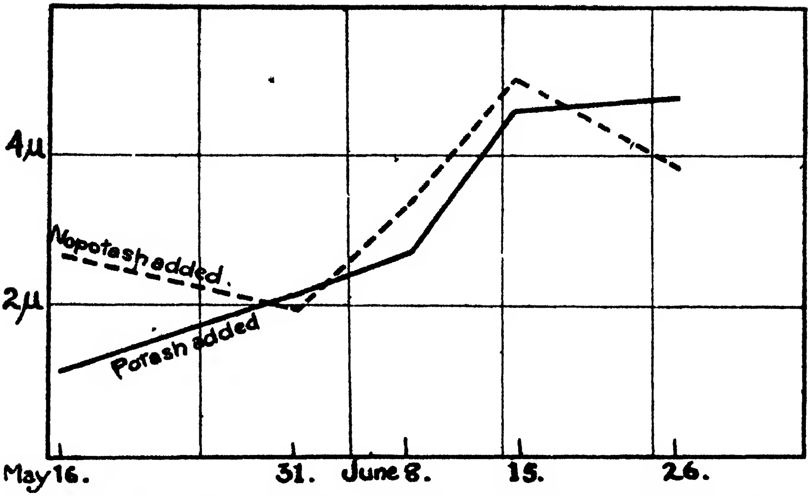

Fig. 18. Mean thickness of walls, sclerenchyma, in presence of added nitrogen.

Full line —— Plot 9, with added potassium salts

Broken line - - - Plot 10, without added potassium salts.

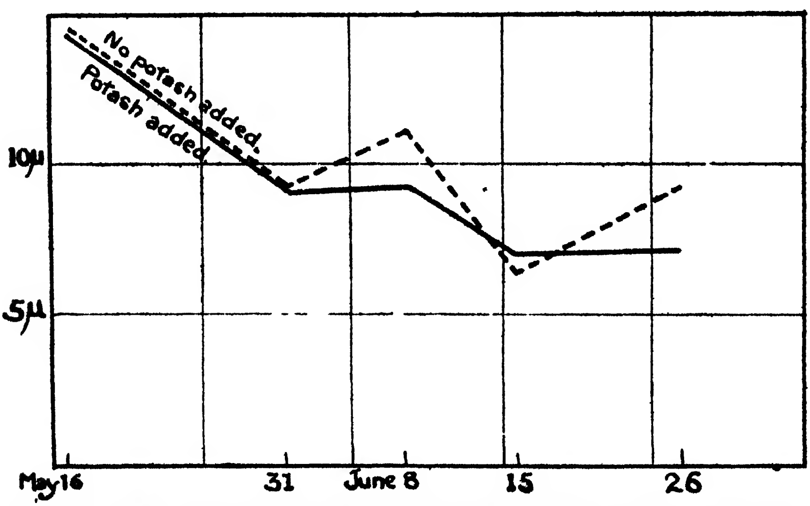

Fig. 19. Mean diameter of lumina, sclerenchyma, in presence of added nitrogen.

Full line —— Plot 9, with added potassium salts.

Broken line - - Plot 10, without added potassium salts.

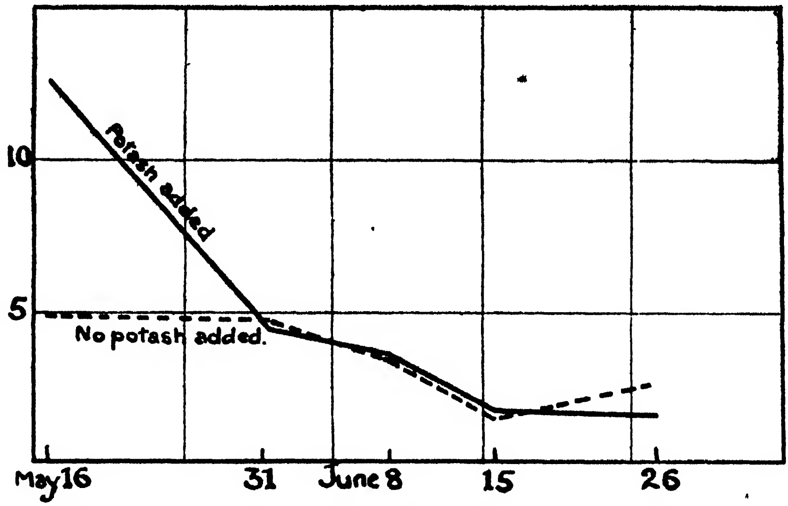

Fig. 20. Mean ratio of lumen diameter to wall thickness, in presence of added nitrogen.

Full line —— Plot 9, with added potassium salts.

Broken line - - - Plot 10, without added potassium salts.

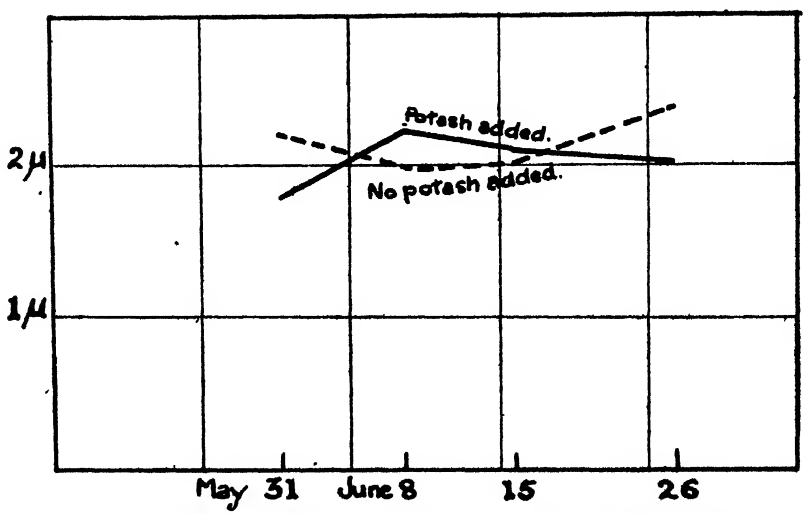

Fig. 21. Mean thickness of walls, xylem, in presence of added nitrogen.

Full line —— Plot 9, with added potassium salts.

Broken line - - - Plot 10, without added potassium salts.

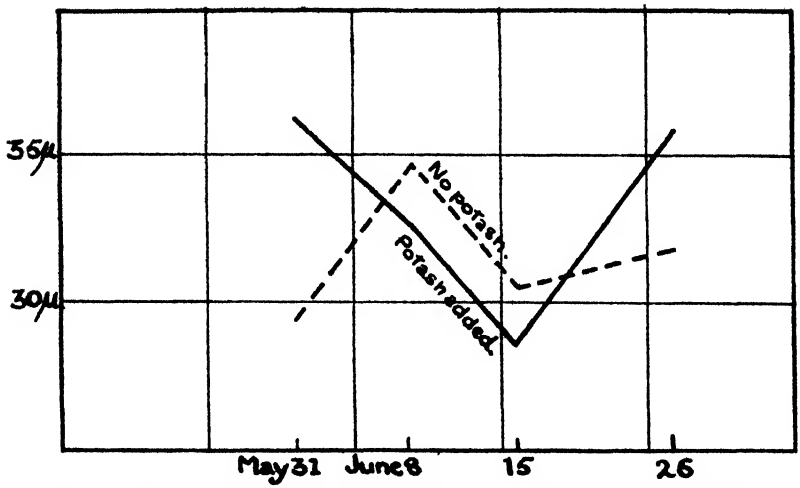

Fig. 22. Mean diameter of lumina, xylem, in presence of added nitrogen.

Full line —— Plot 9, with added potassium salts.
Broken line - - - Plot 10, without added potassium salts.

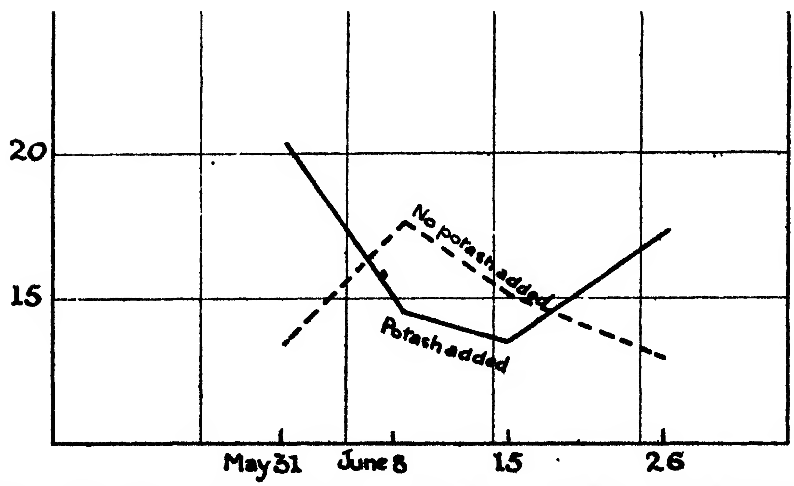

Fig. 23. Mean ratio of lumen diameter to wall thickness, in presence of added nitrogen.

Full line —— Plot 9, with added potassium salts.
Broken line - - - Plot 10, without added potassium salts.

(*Received May* 13*th*, 1919.)

INFLUENCE OF MINES UPON LAND AND LIVESTOCK IN CARDIGANSHIRE.

By J. J. GRIFFITH.

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TABLE OF CONTENTS.

During the years 1908-1913 inquiries with reference to the infertility of soils were frequently received from farmers in various parts of Cardiganshire. Upon examination it was found that the primary cause of infertility in most of the cases was the presence in the soil of appreciable quantities of lead. At first it was thought that only a few isolated spots were thus affected. Further investigation, however, revealed the fact that a relatively large area of what should be naturally the best land of North Cardiganshire was suffering from the same cause, and that a considerable number of farmers were of opinion that both their soils and their live stock suffered from "lead poisoning." Consequently, it was thought advisable to make a survey of the affected land and to investigate possible remedial measures.

It appears that attention has been drawn on various occasions in the past to the undesirable effects of mine wastes upon Cardiganshire soils.

Gwallter Mechain[1] writing in 1815 states: "During conversation with a Cardiganshire gentleman he observed how niggardly Nature had bestowed her blessings on his native county. We endeavoured to frame an apology for Nature; and, among other instances of her liberality, mentioned the silver and lead mines. 'O,' he exclaimed, 'that is a curse, and not a blessing; the mines enrich a person or two in an age, and entail poverty on hundreds for generations to come.' 'The waters from the mines,' he added, 'spread sterility over the adjacent fields, and kill all the fish in the rivers.'"

Again, the Rivers Pollution Commissioners in a report published in 1874, p. 15, indicate that, of all the lead mining districts in Great Britain, Montgomeryshire and more especially Cardiganshire were the only two areas where land and live stock suffered injury to any marked extent—the valleys of the Ystwyth, Rheidol, Clarach and Dyfi being particularly mentioned.

Still another record of this trouble is to be found in the "Report of the Royal Commission on land in Wales, 1896." On April 28th, 1894, a witness made the following statement: "Adjoining the river there was a meadow on which we kept ten cows and a bull. About 45 years ago the river, which was then strongly impregnated with lead from the mines, overflowed this meadow and left a mineral deposit which made it not only useless, but dangerous for the horses, and we could only keep three or four cows on it, and the butter of these cows would be quite unsaleable."

It is thus evident that many generations of farmers have had to suffer serious losses as a consequence of the working of the lead mines.

SOURCES AND AGENCIES OF CONTAMINATION.

It is found that there are a number of ways in which mine refuse may be carried on to the land, and also lead to the poisoning of farm live-stock.

1. By surface drainage water from the heaps of débris at the mines.

In the neighbourhood of most mines of importance enormous heaps of waste material accumulate. Some of these heaps consist of rock material in the form of coarse stones which are considered to contain too little ore to be profitably worked. The other heaps consist of the so-called "sand" and "slime" representing the fine waste material left after the separation of the ore at the dressing-floors and also the sediment

[1] Gwallter Mechain's *Tour through South Wales*, 1, p. 80.

which accumulates in the "slime" or "catch" pits. Even with the most careful management and the employment of modern machinery some of the ore finds its way into these waste heaps. In the case of some mines, however, it is found that the waste heaps contain appreciable quantities of ore-laden matter. At the present time some of them are found to contain enough ore to make further extraction profitable.

In the fresh heaps the lead is usually present as sulphide and the clean drainage water from them is found to be practically free from lead. After the heaps have been exposed to the action of air and water for some years, however, it is found that the drainage water from them is impregnated with lead and other metalliferous substances. A sample of soil from a field near Talybont, contaminated with surface water from the wastes of a disused mine, was found to contain ·11 per cent. of lead.

2. Sand, and more particularly slime, are blown from the heaps by the dry east winds. Fields situated on the western sides of the mine heaps are almost invariably affected in this way, in some cases, e.g. at Cwmsymlog, for a distance of at least a mile. This effect is readily perceived when there is a strong east wind and the ground is covered with a layer of snow.

The following are results of analyses of soil samples taken on the western and eastern sides of mine refuse heaps. The samples were taken on land which as far as could be observed had been contaminated by wind action only.

Mynydd Gorddu Mine. Sample from		Cwmsymlog Mine. Sample from	
(*a*) West side	(*b*) East side	(*a*) West side	(*b*) East side
Lead ·08 %	Lead-trace	Lead 0·9 %	Lead, nil

3. Where mine wastes have been utilised to construct and repair roadways, some of the material may, in course of time, be washed over the adjoining land especially where the roads are not protected by a bank or ditch.

A striking example of this effect is to be seen at a farm near Brynarian Mine. The roadway leading up to the farm goes through the centre of a field situated on a slope. Above the roadway the soil is fertile and free from toxic substances. On the lower side of the road there is a strip of land ranging in width from 10 to 20 yards where the vegetation presents the usual appearance of that growing on lead poisoned soils.

Analysis of soil samples showed

(*a*) Soil above road to be free from lead.

(*b*) Soil below road to contain ·12 per cent. of lead.

4. Artificial waterways (leats), some of them miles in length, made to conduct washing water from one mine to another. In many places these leats almost follow the contour lines and thus the motion of the water is very slow so that there is a great tendency for the suspended slime to settle down. The bed of the leat is thus gradually raised and the slimy water overflows and contaminates the land which lies lower down the slope. See photograph of leat (photograph "W") and also analytical results given in Table IV.

5. Water drawn from polluted rivers and carried along artificial water-courses towards various mills and farms to provide water-power has in many instances poisoned stock and contaminated adjoining fields. A sample of the sediment from a mill pond in the Leri valley was analysed and found to contain 1·74 per cent. of lead.

6. Water running out of mine levels, both active and disused, is often highly contaminated with lead, zinc and other metallic substances. A special feature of this means of contamination is that the poisonous metals are in solution in the water. Consequently the injurious effects upon land and stock are much more pronounced and immediate. A sample of the water flowing from an old level near Cwmystwyth was found to contain in solution ·3 part of lead per 1,000,000. This water was suspected of having poisoned a number of sheep which had been grazing on adjoining land.

7. In the case of farms situated in the immediate vicinity of mine wastes the animals themselves, especially poultry, pick up and consume the poisonous substances direct from the heaps. In fact, on many farms it is well recognised that poultry cannot be kept in places where they have any access to the mine heaps.

8. Several cases are on record where farmers having used material obtained from polluted ditches for making composts have lowered the fertility of the land. Also, sand and gravel obtained from the mines and utilised for making building mortar, covering pathways and other purposes, have often led to the poisoning of farm animals.

9. Rivers in times of floods have been the means of depositing enormous quantities of mine refuse upon the land, more especially in the lower river valleys where the conditions are favourable to sedimentation. Undoubtedly this is the manner in which most of the land has been affected. The great majority of the mines are situated on river banks in the upper reaches of the valleys in the eastern portion of the county (see map "A"). It thus follows that most of the land in the river valleys all the way to the sea is liable to be affected.

The river valleys which have suffered most are those of the Ystwyth, Rheidol and Clarach. The injurious effects are very marked also in the upper reaches of the Teifi, but below Tregaron the Teifi valley does not appear to have suffered much. Along the banks of the Leri, Clettwr, Einon and Afon Ddu only relatively small areas of land have been seriously affected.

There appears to be a tendency to attribute the pollution of the rivers to the waste water which flows into them from the dressing floors of active mines. It is true that this is a contributory cause, but, consistent with retaining economic working conditions for the mines, it seems to be almost unavoidable.

But most of the Cardiganshire rivers may be polluted even after all mining operations on their banks have ceased. In the neighbourhood of many of the mines where much "washing" has been done enormous heaps of "sand" and "slime" are left close to the banks of the rivers. No precautions have been taken to prevent the heavy rains, so common in the hilly districts, from washing down large quantities of this poisonous mine refuse to the rivers every year. See Photograph "U."

It must also be borne in mind that the river beds are now covered with a layer of mine refuse ranging in depth from a few inches to several feet. At every flood time some of this is brought into suspension and spread over the adjoining fields. See Table IV.

It should be noted, however, that this sediment is probably, to a considerable extent, to be accounted for by the fact that years ago when there were no state regulations with reference to the disposal of mine refuse, it was customary at many of the mines to tip the whole of the "slime" and "sand" directly into the rivers.

Influence of Mine Refuse upon Crops.

The intensity of the deleterious influence upon land varies from slight effects hardly apparent on a mere casual observation to complete sterility where the soil remains absolutely bare, not even producing weeds. Between these extremes there are to be seen all degrees of unproductiveness according to the amount and nature of injurious matter present. The harmful effects are observable on all farm crops, both quality and bulk of produce being unfavourably affected, though the effect is more marked with some crops than others.

(*a*) Effects upon grass land.

Pastures which are affected usually present a very characteristic appearance, especially during certain periods of the year. In most

cases contamination of the soil leads to a very considerable change in the type of vegetation. Speaking generally, bent-grass (*Agrostis vulgaris*) predominates and where the harmful effect is very pronounced the herbage may consist entirely of bent. Where the situation is somewhat damp there is a tendency for Yorkshire fog (*Holcus lanatus*) to take the place of bent to some extent, and even *Molinia coerulea* may flourish in some comparatively wet situations although it does not appear on the adjoining unaffected land. Another peculiarity of these affected pasture lands is the almost universal absence of clovers even in areas where contamination is but comparatively slight. It appears that clovers are less resistant to the evil influences of mine refuse than almost any plant entering into the composition of the herbage on normal soils. Sheep's fescue (*Festuca ovina*) flourishes relatively well on soils which are only slightly affected. The presence of certain weeds, e.g. *Viola lutea* and sea campion (*Silene maritima*), also frequently serves to distinguish the affected areas.

The general appearance of affected pasture land is such as to enable one to draw the line of demarcation between it and the non-affected land with comparative ease, the herbage of the former being scanty, of poor quality, and, towards the autumn, showing proportionately much more withered inflorescence.

In order to illustrate the results of my observations with reference to the effects upon grass land, Mr R. G. Stapledon kindly carried out a botanical analysis of the herbage on a field which is quite typical of the affected land. In Table I, *A* represents the herbage on unaffected land, while *B* and *C* show the composition of the herbage on affected land in the same field.

TABLE I.

	A. Unaffected	*B.* Slightly affected	*C.* Badly affected
Agrostis vulgaris	46	90	100
Cynosurus cristatus	4	—	—
Holcus lanatus	3	·8	—
Festuca ovina	4	2	—
Trifolium repens	23	—	—*
Miscellaneous (chiefly Plantago lanceolata)	20	—	—
Number inflorescences to 10 readings	76	136	280
Weight of 100 inflorescences (gms.)	1·3	4·1	13·9
Inflorescences : grass	100 : 937	100 : 522	100 : 218

(*b*) Effects upon arable land crops.

When affected land is under the plough it seems to do even worse than when in grass. In fields of roots and of cereal crops it is a common thing for one to see absolutely bare patches which when the fields are in grass would be covered by some type of vegetation. The seeds of cereals, etc., germinate satisfactorily, and the seedlings flourish for a time. Then the plants turn a reddish colour and subsequently remain in a stunted condition or perish altogether. When such land is afterwards put down to grass, the herbage for the seed hay, though always free from clovers, is often fairly satisfactory as far as the grasses are concerned, but in the second, or at most the third year the land is overrun with bent once more. See Photographs "P," "Q," "R," "S" and "T."

Influence of Mine Refuse upon Animals.

Practically all classes of farm stock are liable to suffer when grazing on affected land or when fed with crops raised on poisoned areas. Sheep, horses and poultry suffer most, the effects upon cattle being as a rule less pronounced.

Sheep grazing on affected areas often abort and even the lambs which survive do not thrive well. The ewes begin to waste away when three years old and, consequently, have to be disposed of although under favourable conditions they would have been kept for another year.

Horses, when affected, at first seem to get into good condition but later suffer from diarrhoea, then follows a stiffness of the limbs and paralysis, and, what is most characteristic of all, they ultimately become "broken-winded." The experience of many farmers tends to indicate that horses reared on affected land suffer most and often die before they are three, or at most four, years old, whereas adult horses brought in from unaffected farms seem more capable of resisting the effects.

Complaints regarding injury to cattle are made only in a few isolated districts, at least as far as fatal effects have been reported. However, even in districts where cattle do not die from the effects there seems to be a consensus of opinion that they do not thrive as well when they have access to poisoned soils; they are also often found to exhibit indications of the effects when forced to take more than the usual amount of exercise. For instance, when they are walked to a fair they seem to get into an exhausted state and lag behind after proceeding a few miles.

Poultry seem to be very sensitive to the influence of mine refuse. When they have access to mine heaps, polluted brooks or contaminated

land they lay badly and tend to produce shell-less eggs, and many of them perish when the effects become more marked.

Many farmers are very reluctant to admit their troubles with livestock because dealers often endeavour to avoid purchasing animals from affected farms.

In order to show that the loss with livestock is very considerable a few of the cases that have been under observation may be cited.

(*a*) In the spring of 1912 one farmer lost 170 sheep. Both the farmer and the veterinary surgeon, Captain R. D. Williams, M.R.C.V.S., were satisfied that the symptoms were those of lead poisoning. Captain Williams conducted post-mortem examinations and handed me specimens of the livers, which I found to be highly impregnated with lead. I also took some of the sediment left on the herbage where the sheep had been grazing and found the air-dried sample to contain

Moisture	3·12 %
Lead (Pb)	1·36 %

(*b*) During 1913 and 1914 a farmer lost four horses due to "plumbism" as testified by symptoms, post-mortem examination conducted by Captain Williams, and also the presence of lead in liver sample which I received for analysis.

(*c*) Another farmer, during the years 1898 to 1914, lost twenty horses as the result of grazing on contaminated land.

For further information regarding injury to stock see *Journal of the College Agricultural Department*, vol. VIII. article on "Plumbism in North Cardiganshire," by Mr Edward Morgan, M.R.C.V.S., D.V.H.

CAUSES OF INJURIOUS EFFECTS ARISING FROM PRESENCE OF MINE REFUSE IN SOILS.

Although contaminated soils are usually said to be "lead poisoned" it must be borne in mind that many mines in the county contain in addition to lead ore, several other minerals which may prove harmful.

List of Minerals in the Metalliferous Veins of Cardiganshire.

NAME OF MINERAL	RELATIVE ABUNDANCE	REMARKS
Galena (PbS)	Most abundant ...	Containing up to 80 oz. of silver to ton of lead, but usually much less.
Cerussite ($PbCO_3$)	Very small quantity ...	Covering galena where there has been infiltration of water containing carbonic acid.

Name of Mineral	Relative Abundance	Remarks
Pyromorphite ($3Pb_3(PO_4)_2 . PbCl_2$)	Traces ...	Covering galena exposed to air
Blende (ZnS)	Next to galena in abundance	
Hemimorphite or Smithsonite (zinc silicate)	Small quantity	
Calamine (zinc carbonate) ...	Very small quantity	
Iron pyrites (FeS_2)...	Abundant at some mines	Fairly free from arsenic
Marcasite (FeS_2)	Amount not large	
Copper pyrites ($CuFeS_2$)	Workable amount only at few mines	Only traces of arsenic
Malachite ($Cu(OH)_2 . CuCO_3$) ...	Small quantity	
Azurite ($2CuCO_3 . Cu(OH)_2$) ...	Very small quantity	
Manganite (hydrated oxide of manganese)	Occurs only at few mines	
Siderite (carbonate of iron) ...	do. do.	

Composition of Mine Refuse.

In order to ascertain the nature of the mine refuse likely to be carried from the mines on to the land samples were taken of the

(*a*) mine heaps,

(*b*) river waters,

(*c*) sediment from leats and river beds.

The results of the analyses of these samples are given in Tables II, III and IV.

Samples 1 to 11 were taken by an official of the Teifi Board of Conservators with the object of providing information to a Special Committee of the Cardiganshire County Council holding a public enquiry into the destruction of fish life in the Teifi by mine polluted water. These samples were forwarded to me periodically during the summer of 1917 and were analysed as they arrived.

As a result of pollution with mine refuse all fish life has been destroyed in most of the rivers of North Cardiganshire.

Table III shows the nature of mine refuse at its source and Tables II and IV that of the refuse in course of transit on to the land. The following are some of the deductions which may be drawn from the analytical figures in these tables.

(*a*) Lead and zinc compounds appear to be the only metalliferous substances present in such quantities as are likely to inflict injury upon plants and animals. In the mine heaps, and also in the leats and rivers, copper and arsenic are present only in very minute quantities. In a few districts, however, the amount of iron pyrites present is such as may lead to harmful effects upon vegetation.

TABLE II. *Showing amount and nature of impurities in the rivers of the District.*

The figures represent parts per million.

No. of Sample	Name of stream or river	Total Solids	Inorganic Solids	Organic Solids	Lead (Pb)	Zinc (Zn)	Remarks
1	Glogfawr stream	58	39	19	Trace	—	Samples taken when water was relatively clear.
2	Marchnant	47	31	16	Trace	—	
3	Nantfynaches	64	42	22	·05	—	
4	Nantfynaches	392	332	60	·2	—	Samples taken when streams appeared turbid and more highly polluted.
5	Tributary to Marchnant ...	220	187	33	·1	—	
6	Marchnant	121	91	30	Trace	—	
7	Esgairmwyn stream	458	382	76	10·0	—	Samples taken after heavy rain and the streams had received much surface water from mine heaps.
8	Marchnant	157	114	43	·4	—	
9	Stream above mines ...	70	37	33	Nil	—	Sample to show nature of water before it enters mine.
10	Teifi	41	24	17	Trace	—	Sample when river not very turbid.
11	Teifi	67	45	22	·1	—	Sample when river appeared polluted.
12	Ystwyth	46	—	—	Nil	—	Sample taken when the water was clear.
13	Ystwyth	856	—	—	·04	·1	Sample taken at time of flood.
14	Cwmbrwyno	342	—	—	·9	Trace	Stream running through mine refuse.
15	Erwtomau	370	—	—	Trace	·3	Stream from active mine.
16	Castell...	262	—	—	Trace	·2	Stream with effluent from active mine.
17	Rheidol	51	—	—	Nil	—	Water clear at sampling time.
18	Rheidol	911	—	—	·1	·2	Sample taken at time of flood.
19	Rheidol	432	—	—	·5	Trace	Mine refuse heaps on bank close to position of sampling.
20	Rhydbeddau (Upper Clarach)	640	—	—	1·6	Trace	Sample taken where mine refuse had been thrown in.
21	Leri	224	—	—	·2	Trace	Sample taken where mine effluent enters.

In most of the water samples examined practically the whole of the lead and zinc were present in suspension. In samples 8 and 20, however, a small quantity of the lead was present in solution and in sample 7 it was mainly in solution. Sample 20 contained a trace of copper. All the samples were free from arsenic.

TABLE III. *Composition of Mine Refuse Heaps.*

Percentages in air-dried samples.

Description.	Sand.	Sand.	Sand.	Slime.	Slime.	Slime.	Slime.	Slime.	Slime.	Slime.
Mine.	Frongoch	Level Fawr	Goginan	Frongoch	Level Fawr	Llywernog	Goginan	Darren	Cwmsymlog	Henhafod
Reference number.	1	2	3	4	5	6	7	8	9	10
Mechanical analysis:										
Moisture	·3	·4	·4	·5	·5	·6	·5	·4	·2	·4
Loss on ignition	1·6	4·2	2·0	1·4	3·4	1·9	2·7	3·5	1·8	—
Fine gravel	10·3	12·8	11·0	1·4	2·6	2·3	1·0	2·7	1·6	—
Coarse sand	71·9	69·1	73·7	24·7	35·9	5·1	27·4	7·6	20·3	—
Fine sand	8·4	12·0	11·1	42·0	30·5	42·2	34·7	38·2	44·6	—
Coarse silt	4·8	1·3	1·2	22·9	12·9	32·1	19·7	30·0	20·3	—
Fine silt	·7	·6	1·0	3·3	12·5	13·4	8·6	12·2	8·1	—
Clay	Trace	Trace	Trace	Trace	1·5	1·0	1·2	1·4	1·4	—
Carbonate (calc. as $CaCO_3$)	,,	Nil	,,	Nil	Nil	Nil	Nil	Nil	Trace	39·1
Total	98·0	100·4	100·4	96·2	99·8	98·6	95·8	96·0	98·3	—
Chemical analysis:										
Lead (Pb)...	·42	·24	·50	1·80	15·60	·09	·67	1·1	·36	1·62
Zinc (Zn)	1·07	Trace	Trace	3·68	Trace	2·18	·11	Trace	Trace	Trace

Samples 7 and 8 contained small quantities of copper. Sample 8 contained a trace of arsenic. In the vein at Henhafod Mine the matrix is mainly calcite, but the composition of the refuse heaps shows that at the other mines it is mainly quartz. It is not to be assumed that refuse heaps as a whole contain as much lead and zinc as is indicated in the table. In some cases, e.g. Sample 5, samples were taken where the heap appeared most metalliferous, and where that portion of the heap was so situated that it could be easily washed by rain water to the adjoining river and, therefore, lead to contamination of soils.

TABLE IV. *Composition of Sediments from Rivers and Mine Leats.*

Percentages in air-dried samples.

Locality.	Ystwyth river	Rheidol river	Clarach river	Frongoch leat	Cwm Merfin leat
Reference number.	1	2	3	4	5
Mechanical analysis:					
Moisture	·2	·4	·2	·5	·6
Loss on ignition ...	1·2	1·6	2·8	3·0	2·4
Fine gravel	24·8	19·0	16·9	1·1	1·0
Coarse sand	56·2	58·3	60·2	18·2	16·1
Fine sand	10·1	12·0	10·3	42·3	46·3
Coarse silt	5·8	7·1	7·9	28·1	27·2
Fine silt	1·2	1·8	·9	6·0	4·0
Clay	Trace	Trace	Trace	·8	1·9
Carbonate (calc. as $CaCO_3$)	Trace	Nil	Nil	Nil	Nil
Total	99·5	100·2	99·2	100·0	99·5
Chemical analysis:					
Lead (Pb)	·11	·10	·20	1·56	2·67
Zinc (Zn)	·18	·12	Trace	1·97	·10

(*b*) The clear waters of the rivers are fairly free from poisonous ingredients. But the suspended matters in both leat and river waters often contain relatively large quantities of lead and zinc.

(*c*) Occasionally some of the streams contain considerable quantities of the poisonous metals in the form of soluble compounds (see note, Table II). This happens more especially when the rainfall is heavy after a long period of drought. Some of the mine heaps are situated on peaty land. The sourness of the surrounding land may assist the process of solution of the lead in the sediment washed on to its surface from the heaps. Consequently surface drainage from such land may contribute largely to the pollution of the adjoining river with soluble lead compounds at the time of the first heavy rain after a period of drought. This may be the reason why, according to the evidence of some farmers, vegetation on contaminated soils is more poisonous to stock after the first flood subsequent to drought than at any other time.

(*d*) The mechanical composition of the mine "sand" and of the sediment of polluted rivers is such as may lead to unfavourable changes in the physical properties of light soils.

(*e*) The sediment in the leats is much finer than that found in the river beds. It approaches the mine slime in mechanical composition. It is found, however, to contain a much higher percentage of the poisonous metals.

TABLE V. *Analyses of Soils showing Effects of Leats and Polluted Drainage Water.*

Locality.	Cwm Merfin				Cwmsebon				Frongoch		Dolfawr Rheidol valley		Caegynon Rheidol valley	
Description.	Unaffected		Affected		Unaffected		Affected		Unaf-fected	Af-fected	Unaf-fected	Af-fected	Unaf-fected	Af-fected
Reference number.	1		2		9		10		3	4	33	34	31	32
	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Sub-soil	Soil	Soil	Soil	Soil	Soil	Soil
Mechanical analysis:														
Moisture	3·5	3·1	2·8	3·2	2·8	2·2	2·1	1·8	1·5	1·8	1·2	1·5	2·2	2·0
Loss on ignition ...	12·3	8·2	11·8	7·8	12·3	8·7	11·0	7·4	10·8	9·4	6·8	9·4	9·8	10·1
Fine gravel	11·5	13·6	8·0	12·9	15·8	18·9	12·6	16·7	19·6	18·4	3·1	4·0	16·4	13·8
Coarse sand	9·3	10·2	7·9	10·6	8·8	8·1	7·7	10·3	10·2	11·5	10·1	10·1	15·0	15·0
Fine sand	12·0	12·2	14·6	11·7	8·1	9·3	11·7	9·2	7·5	10·4	12·4	10·8	8·4	11·9
Coarse silt	11·9	11·2	15·5	12·6	11·4	11·0	15·9	12·1	14·1	15·8	29·9	25·8	20·7	31·4
Fine silt	29·1	29·0	30·2	29·7	31·0	30·2	31·1	30·9	27·1	24·0	26·9	27·2	19·3	9·2
Clay	9·3	9·7	7·2	8·6	7·9	9·0	6·8	7·7	9·2	6·7	9·5	10·0	6·8	5·7
Carbonate (calc. as $CaCO_3$)	Nil	Nil	·1	Trace	Trace	Nil	Trace	Trace	Nil	Nil	Nil	Nil	Nil	Nil
Total	98·9	98·2	98·0	97·1	98·1	97·4	98·9	96·1	100·1	98·0	99·9	98·8·	98·6	99·1
Chemical analysis:														
Nitrogen	·361	—	·296	—	·350	—	·279	—	·304	·216	·240	·241	—	—
Total potash (K_2O) ...	·662	·784	·591	·680	·702	—	·724	—	·583	·612	·498	·516	—	—
Available potash (K_2O)	·032	—	·029	—	·028	—	·026	—	·027	·030	·019	·023	—	—
Lead (Pb)	Nil	Nil	1·70	·26	Nil	Nil	·82	·06	Nil	·92	Nil	·35*	Nil	·23
Zinc (Zn)	Nil	Nil	Trace	Trace	Nil	Nil	·07	Trace	Nil	1·20	Nil	Trace†	—	—

All samples referred to in Table V were taken from fields in which the herbage showed that the injurious effects were very marked.

* 0·56 in subsoil. † Trace in subsoil.

INFLUENCE OF MINE REFUSE UPON THE COMPOSITION OF SOILS.

The analyses of a number of soils are given in Tables V, VI and VII. The mechanical composition is given throughout. The chemical composition is included only in so far as it presents any points of contrast between affected and unaffected soils.

DEDUCTIONS FROM TABLE V.

(*a*) There is no appreciable alteration in the mechanical composition of the soils. Usually there is only a slight increase in the proportion of fine sand and coarse silt. This change is not likely to have any serious effect upon fertility.

(*b*) With regard to changes in chemical composition it is usually found that the nitrogen content is lowered as a result of contamination.

(*c*) The amount of toxic metals, lead and zinc, present is high and much higher than in soils affected by river water. See Tables VI and VII.

DEDUCTIONS FROM TABLE VI.

(*a*) Contamination has brought about a very considerable change in mechanical composition. The coarse grade of particles have been increased to such an extent that the texture of the soils is much too open. See samples Nos. 7, 8 and 27.

(*b*) The store of plant food is lowered, both with regard to nitrogen and potash.

With reference to this point it is to be noted that apart from the alluvial soils of the river valleys the soils of Cardiganshire are very rich in potash and deficient in phosphate and lime, and, generally speaking, are remarkably uniform in their chemical composition. The alluvial soils of the river valleys, on the other hand, are not as rich in potash as the sedentary and glacial soils of the country. As it is mainly the soils in the river valleys that have been affected it is evident that the lowering of the percentage of potash may be one cause of their infertility.

(*c*) The toxic metals, lead and zinc, are present in smaller quantities than in leat affected land. Contrast Table V.

DEDUCTIONS FROM TABLE VII.

(*a*) Contamination has not greatly affected the mechanical composition of these soils. In this respect there is a resemblance to leat affected soils; cf. Table V.

(*b*) The amount of toxic metals may or may not be high.

TABLE VI. *Analyses of Soils affected by River Water where Land is unprotected by embankments.*

Reference number.	5	6	7	8	26	27	11	12	24	25
Locality.	Goginan.	Goginan.	Goginan.	Goginan.	Penllwyn. Rdl. vall.	Penllwyn. Rdl. vall.	Llanilar. Yst. vall.	Llanilar. Yst. vall.	Mont-gomery.	Mont-gomery.
Description.	Unaffected	Affected S.	Affected B.	Affected V.B.	Unaffected	Affected B.	Unaffected	Affected B.	Unaffected	Affected S.
Mechanical analysis:										
Moisture	3·0	3·1	·1	·5	1·8	·3	1·2	1·3	4 3	2·3
Loss on ignition ...	11·5	10·8	4·3	3·5	11·1	3·7	6·0	7·3	10·3	9·3
Fine gravel ...	10·5	5·3	·2	10·0	20·6	25·6	·2	·6	1·9	1·7
Coarse sand ...	11·6	15·0	51·2	70·6	21·0	42·0	20·7	6·7	1·5	4·0
Fine sand ...	9·7	15·3	28·9	12·2	19·1	15·3	29·3	48·9	9·7	31·7
Coarse silt ...	18·8	19·7	7·0	1·4	19·4	8·2	19·2	18·7	23·6	25·3
Fine silt ...	26·5	22·8	3·8	1·2	3·5	3·1	16·4	11·3	34·8	16·5
Clay	8·6	7·4	2·0	Trace	1·9	·8	4·5	4·1	12·7	8·1
Carbonate (calc. as $CaCO_3$) ...	Nil	Nil	Nil	·1	Nil	Nil	Nil	Nil	Trace	Nil
Total	100·2	99·4	97·5	99·5	98·4	99·0	96·3	98·9	98·8	98·9
Chemical analysis:										
Nitrogen	·403	·301	·117	·090	—	—	·202	·186	—	—
Total potash (K_2O)	·496	·490	·318	·286	·314	·222	·597	·316	—	—
Available potash (K_2O)	·022	·017	·014	·010	·020	·009	·033	·011	—	—
Lead (Pb)... ...	Nil	Trace	·09	·23	Nil	·09	Nil	·13	Nil	0·8
Zinc (Zn)	Nil	Nil	Trace	Trace	Nil	·03	Nil	·16	—	—
Lead in subsoil ...	Nil	Trace	—	·13	Nil	·07	Nil	—	Nil	·06
Zinc in subsoil ...	Nil	Nil	—	Nil	Nil	·06	Nil	—	—	—

S. = Slightly affected. B. = Badly affected. V.B. = Very badly affected—soil remaining bare.

TABLE VII. *Analyses of Soils affected by River Water where Land is more or less protected by embankments.*

Reference number.	13	14	20	21
Locality.	Tancastell. Yst. valley.	Tancastell. Yst. valley.	Cwmcoedwig. Yst. valley.	Cwmcoedwig. Yst. valley.
Description.	Unaffected	Affected B.	Unaffected	Affected S.
Mechanical analysis:				
Moisture	1·4	2·0	2·1	1·8
Loss on ignition	6·8	7·4	15·4	13·2
Fine gravel	1·6	·3	5·6	4·9
Coarse sand...	14·5	7·0	16·1	18·1
Fine sand	33·0	36·3	24·3	27·0
Coarse silt	22·4	27·1	20·3	16·6
Fine silt	15·8	14·6	10·2	12·4
Clay	5·1	3·7	5·0	6·1
Carbonate (calc. as $CaCO_3$)	·1	Trace	Nil	Nil
Total	100·7	98·4	99·0	100·1
Chemical analysis:				
Nitrogen	·251	·198	—	—
Total potash (K_2O) ...	·871	·814	—	—
Available potash (K_2O) ...	·042	·040	—	—
Lead (Pb)	Nil	·32	Nil	Nil
Zinc (Zn)	Nil	·10	Nil	Nil
Lead in subsoil	Nil	·09	Nil	·01
Zinc in subsoil	Nil	·02	Nil	Trace

B. = Badly affected. S. = Slightly affected.

FURTHER DEDUCTIONS FROM TABLES V, VI AND VII.

(*a*) Lead occurs in practically all affected soils. Zinc also is present in most of them. Copper and arsenic were not detected in any of the soils dealt with, except in samples from a few small strips of land close to the leats and refuse heaps of a few mines which were found to contain small quantities of copper.

(*b*) There is usually much more of the toxic metals in the soil than in the subsoil. But in some cases the subsoil contains more than the soil. Sample 21, Table VII, is an instance of contaminated land where lead and zinc are present in the subsoil but not in the soil.

(*c*) There had been no undesirable increase of fine silt in any of the samples examined.

To sum up, a consideration of the figures given in Tables V, VI and VII suggests that admixture with mine refuse may possibly lower soil fertility in the following ways:

1. By the toxic action of lead and zinc—Tables V, VI and VII.
2. By unfavourably affecting the mechanical composition—Table VI.
3. By lowering the percentage of plant food in the soil.
   (*a*) Decrease of potash—Table VI.
   (*b*) Decrease of nitrogen—Tables V, VI and VII.

Removal of Natural Soil.

In many areas a much greater alteration of composition has been produced than would result from mere contamination of the natural soil. The partial, or in some cases even complete destruction of the herbage, enabled the heavy rains on the hill slopes, and the river floods in the valleys, to wash away a good deal of the fine soil. Consequently the soil has become much more stony. For instance, on the slope above Frongoch mine, where wind blown material from the heaps has proved very destructive to vegetation, it was found that the soil contains 85 per cent. (approx.) of stones, whereas in the case of adjoining land where the herbage had been but slightly affected the soil contained only 58 per cent. of stones. See Photograph "O." In the case of the river valleys the effect has been even greater. As a result of the destruction, by the poisoned water, of the vegetation which binds the soil on the grassy borders of a river, the containing banks are weakened and the course of the river is often diverted. The flow of the river may be changed also by the accumulation of mine débris in the river bed. In addition, the floods have washed away all the natural soil of adjoining land. Consequently, in these situations at present we have a soil consisting almost entirely of mine slime and sand, resting upon shingle beds or river gravel. In both the Ystwyth and Rheidol valleys there are large tracts of land of this type.

Formation of New Surface Soil.

In some situations the floods have deposited the mine refuse on the surface of the original soil, thus forming an artificial soil with the natural soil acting as subsoil. This is well exemplified by the following analytical results obtained with samples taken near Capel Bangor in the Rheidol valley. It will be noted that the mechanical composition of the subsoil of affected land is very similar to that of the soil of unaffected land.

	Affected land.		Unaffected land.
	Soil	Subsoil	Soil
Moisture	2·0	2·4	2·5
Loss on ignition	6·3	8·6	10·9
Fine gravel .	4·6	25·2	22·1
Coarse sand.	32·9	22·3	19·4
Fine sand	44·8	19·8	20·4
Coarse silt	5·1	14·1	15·7
Fine silt	1·2	4·3	4·5
Clay ...	·3	1·9	2·3
Carbonate	Trace	Nil	Trace
Total	97·2	98·6	97·8
Stones	Negligible	25	21

Effects upon Physical Properties of Soils.

Pastures on affected land almost invariably present an appearance which is very similar to that of herbage growing on soils liable to suffer from drought. It is reasonable to suppose that one cause is a deficiency of moisture following an alteration in the mechanical composition of the soil as shown in Table VI. But this parched appearance is also found in pastures which have been contaminated but not altered in mechanical composition. It was therefore considered desirable to determine the water content of affected soils in dry weather. This was carried out by taking a series of samples along a line stretching across affected and unaffected land. The samples were packed in air-tight tins and the percentage of moisture determined with as little delay as possible. Table VIII shows the results obtained with samples taken at various dates during the summer months of 1914.

Sections D and E of Table VIII show that contamination by means of polluted river water may diminish the water-retaining capacities of soils very considerably. This unfavourable influence is undoubtedly a factor which affects the herbage very appreciably. As already shown in Table VI contamination by means of river water often affects the mechanical composition of soils very unfavourably. This alteration of mechanical composition does not always lower the capacities of affected soils to retain water. For example, on flat land in some of the river valleys the permanent water table is near the surface and, consequently, the affected soils, although they are much more open in texture, contain nearly as much moisture as the unaffected soils even in very dry weather.

Sections A, B and C of Table VIII indicate that soils contaminated by means of leat water and surface drainage water have not been unfavourably affected as regards their capacities to retain water. But as already stated, the herbage on this class of affected soil also appears very liable to suffer from drought. This may be due to circumstances which diminish the water-absorbing capacity of plants growing on affected land. For instance, root development is very limited; see Photographs "E" and "J." Also the roots are observed to be restricted to the surface soil especially if the lower layers of the soil and the subsoil contain much lead or zinc.

TABLE VIII. *Showing Percentage Moisture in affected and unaffected soils.*

U. = Unaffected. S. = Slightly affected. B. = Badly affected.

Reference Nos. of samples given in brackets.

	Locality and type of crop	Date of Sampling	Moisture %									Means of pollution	
A			U. M(1)	B. M(2)	U. M(3)								A
	Frongoch (bare headland of turnip field)	27.6.14	20·04	18·15	20·21							Overflow from leat	
		5.9.14	20·81	21·92	23·06								
B			U. M(10)	B. M(11)	U. M(12)	U. M(13)	B. M(14)	U. M(15)	U. M(16)	B. M(17)	U. M(18)		B
	Cwm Merfin (pasture)	29.6.14	17·11	16·30	18·06	22·10	19·20	21·3	19·08	20·76	21·13	Leat water	
		8.9.14	15·89	21·92	19·81	20·98	19·90	—	20·15	22·48	—		
C			U. M(4)	S. M(5)	B. M(6)	U. M(7)	S. M(8)	B. M(9)					C
	Dolfawr (Rheidol valley) (oat crop)	27.6.14	17·21	16·44	18·01	19·09	18·87	20·26				Surface drainage and possibly also polluted water from levels	
D			U. M(19)	B. M(20)	B. M(21)	B. M(22)	U. M(23)	U. M(24)	S. M(25)	U. M(26)	U. M(27)		D
	Goginan (pasture)	15.7.14	17·58	7·21	8·46	8·13	19·16	17·40	11·62	19·01	16·49	River water	
		7.9.14	13·62	8·17	7·78	6·26	16·58	15·51	—	—	—		
E			U. M(28)	B. M(29)	U. M(30)								E
	Ystwyth valley (pasture)	15.7.14	20·43	14·59	21·32							River water	
		7.9.14	18·10	9·87	20·64								

Note. All samples were taken in very dry weather when vegetation on the light soils of the district showed signs of suffering from drought. The rainfall during the first week in July, however, may have slightly influenced the samples taken on July 15th.

Lime Requirements.

There appears to be a consensus of opinion among farmers that manuring affected land does not lead to any improvement. Several cases have been observed where a dressing of dung supplemented by artificials including nitrate, phosphate and potash, effected practically no increase in crop production. With reference to the efficacy or otherwise of liming, however, there seems to be no general agreement. Consequently, it was considered advisable to ascertain the lime requirements of mine refuse and also that of contaminated and uncontaminated soils. Some of the results are given in Table IX.

Table IX. *Lime Requirements in tons per acre.*

	Unaffected soil	Affected soil
Soil near Level Fawr (*a*)	1·2	2·6
„ „ (*b*)	1·8	2·8
„ Cwm Merfin (*a*)	2	2
„ „ (*b*)	2·3	2·55
„ Ystwyth valley	·75	·7
„ Frongoch	1·4	2·9
Mine refuse: Bwlch slime	0	
Mine refuse: Level Fawr slime	3·4	
Mine refuse: Frongoch slime	4·8	
Mine refuse: Daren slime	·95	
Mine refuse: Sediment Cwm Merfin leat	1·95	

Except in a few cases, more particularly the neighbourhood of such mines as Level Fawr and Frongoch which contain considerable quantities of pyrites or marcasite, contamination does not increase the lime requirements of soils very materially. For further reference to this matter see Pot Experiments, Table XX, and Field Experiments.

The form in which lead occurs in the soil.

Lead is carried on to the land mainly in the form of galena, and it is found that sulphuretted hydrogen is evolved from all recently contaminated soils when they are digested with strong hydrochloric acid. *Affected soils which have not been contaminated recently are found to be free, or nearly free, from sulphides.* So, in course of time lead sulphide, in the soil, is changed into some other compound or compounds of lead. In the mines, under the influence of water-containing carbonic acid, galena is changed to cerussite. Consequently, one would expect the lead sulphide in the soil to be converted to lead carbonate.

In order to obtain some information on this subject the following experiments were carried out:

(*a*) Drainage waters from affected soils were tested for the presence of lead. Negative results were obtained with the majority of samples. In a few cases only mere traces of lead were found to be present.

(*b*) 10 litres of water were poured on to 10 kgm. of soil, containing 1·3 per cent. of lead and 11·2 per cent. of organic matter, in a tub. The mixture was allowed to stand, with occasional stirring, for three months. At the end of this period the total amount of lead present in solution in the water was found to be ·003 gm.

(*c*) The amount of lead extracted from a number of soils by means of various solvents was estimated. The results are given in Table X.

TABLE X.

Soil	*A*	*B*	*C*	*D*	*E*	*F*
Carbonate (calculated as $CaCO_3$)	·11	Nil	Trace	Nil	Nil	Trace
Total lead (Pb)	1·68	·92	·56	·51	·37	·28
Citric-soluble lead (Pb) ...	·49	·24	·18	·20	·09	·06

No appreciable amount of lead was extracted from any of the samples by treatment with water for seven days. A small trace was obtained in the case of Sample *B*. This sample was found to be slightly sour and probably a small quantity of pyrites was contained in the refuse which contaminated the soil it represents (Frongoch).

If we leave the case of peaty land out of consideration it is obvious that lead is present in the affected soils in forms which are only very slightly soluble in the soil water or in water saturated with carbonic acid. The degree of solubility in 1 per cent. citric acid is quite appreciable, and there appears to be a tendency for the treatment with this solvent to extract about 25 per cent. of the total quantity of lead present. These results, and other considerations already mentioned, may seem to indicate that the lead is present as carbonate. But, in practically all the samples dealt with the amount of soil carbonate present is too low to correspond to the citric-soluble lead. *Consequently it seems highly probable that when lead sulphide is acted upon in a soil deficient in lime, the ultimate result is a combination of the lead with some of the organic compounds of the soil.* It is possible of course that the formation of lead carbonate may be an intermediate step in such a process.

Absorption of Lead by the Plant.

The question which often arises in connection with the poisoning of stock is whether lead is absorbed by the plant. Analysis of the herbage of affected land, and also of the crops grown in pot experiments, almost invariably revealed the presence of lead. In one particular case the following results were obtained with the ash of the herbage of a badly affected area. The sample was divided into three lots, which were dealt with separately as indicated below.

(*a*) Lead in ash of untreated sample	3·63 per cent. Pb.
(*b*) Lead in ash of beaten or thrashed sample	·46 ,, ,, ,,
(*c*) Lead in ash of washed sample	·12 ,, ,, ,,

The presence of lead on the exterior of the plant as a result of splashing and wind action renders the method indicated above unsatisfactory for the purpose of ascertaining whether lead enters into the composition of the plant. This difficulty was eliminated in the following experiments:

(*a*) Swedes grown in an affected field were carefully washed and peeled. The central portions of the roots were dried and ignited. The ash was found to contain ·03 per cent. of lead. The amount of lead contained in the soil upon which the swedes grew varied from ·1 per cent. to ·4 per cent. Pb.

(*b*) Oats were grown in pots containing contaminated soil (with ·41 per cent. Pb.) covered with a layer of sand. The ash of the crop contained ·07 per cent. of lead.

Lead, therefore, enters into the composition of plants growing on affected soils only in very minute quantities. Consequently injurious effects upon animals are likely to be due largely, if not mainly, to the lead in the deposit on the exterior of the plant, or to what may be picked up from the soil itself by close grazing.

POT EXPERIMENTS.

Infertility in mine affected soils may be due to the simultaneous influences of a number of circumstances. Consequently pot experiments were performed with a view to isolating the factors. Additional experiments were carried out in order to ascertain the effects upon different crops and also to discover possible remedial measures to restore fertility.

On account of the liability of distilled water, and of rain water collected under circumstances which involve contact with paint or metal work, to contain metallic impurities, a special collecting surface was

constructed to obtain a store of rain water for watering the pots. This special collector consisted of a wooden framework over which has been fixed a sheet of strong canvas. The water thus collected was tested and found to be free from any trace of metallic impurities injurious to plants.

I. Influence of Metalliferous Minerals upon Fertility.

1. *Effect of Galena upon Oats.*

For this experiment specially selected galena, free from blende and other metalliferous substances, was used. It was ground until it passed through a sieve containing 10,000 meshes to the square inch. The results are given in Table XI.

Variety of seed	Webb's Newmarket.
Time of sowing	May 14th, 1914.
Time of cutting	September 3rd, 1914.

Table XI.

No. of pots	Treatment galena equivalent to Pb	Average weight of straw gm.	Average weight of grain gm.
27 and 28	0	18·2	12·0
29 „ 30	·1 %	14·6	10·9
31 „ 32	·2 %	12·2	10·1
33 „ 34	·4 %	12·8	9·8

In this experiment, therefore, the addition of galena to unaffected soil lowered fertility only to a comparatively slight extent. The development of red pigments, and the other usual peculiarities in the appearance of cereal crops grown in mine affected fields, were observed only in Pots 33 and 34; and even with these the effects were not very pronounced. See Photograph "B."

2. *Influence upon catch crop—Trifolium.*

After removing the oat crop trifolium (crimson clover) was grown as a catch crop. Thirty seeds of trifolium were sown in each pot on October 19th, 1914, and the plants were diminished in number to 20 per pot a month later. During germination, and for about a month afterwards, growth seemed normal and uniform in all the pots. Subsequently a very marked differentiation became evident. The plants growing on the soil to which galena had been added before sowing the oats, acquired a distinctly darker green colour, and the leaves curled

towards the underside to a very marked extent. There was not much difference in the rate of growth at this period. Later on the curling effect gradually disappeared but the leaves slowly developed a reddish hue and afterwards acquired a very dark-red colour. The development of this red colour was accompanied by distinct evidence of the harmful effects of lead upon the growth of the plant. Up to the end of December the rate of growth appeared inversely proportional to the amount of lead added to the soil and to the development of the red colour. See Photograph "C," taken January 5th, 1915.

From this time onwards to the end of March the plants in Pots 31, 32, 33 and 34 gradually became weaker, in fact leaf by leaf they steadily perished towards the end of March. On the other hand, the crop in Pots 29 and 30 slightly improved, and rather suggested some measure of success in an attempt to withstand the unfavourable influence of the lead. See Photograph "D," taken March 25th, 1915.

The crop was cut on April 2nd. Table XII gives the weight of air-dried crop.

Table XII.

No. of pots	Treatment of soil for previous crop	Average weight of trifolium crop (gm.)
27 and 28	Nil	34·6
29 " 30	·1 % Pb	4·3
31 " 32	·2 % Pb	·9
33 " 34	·4 % Pb	·7

It was found also that the roots of the trifolium plants growing in the treated soils were quite abnormal in appearance. They had but few root hairs. They were very stunted and most of them appeared to be in a semi-decayed condition. Nodules, although not abundant, were present on most of the plants. See Photograph "E."

3. *Influence upon second crop of Oats.*

In order to ascertain whether lead mixed with the soil in the form of galena may, as a result of chemical changes, in the course of time become more harmful a second crop of oats was grown in Pots 27 34. The results are given in Table XIII.

Time of sowing	April 14th, 1915.
Time of cutting	August 23rd, 1915.

A dressing of complete manure was given to all pots.

TABLE XIII.

No. of pots	Treatment in 1914	Weight of straw (gm.)	Weight of grain (gm.)
27 and 28	Nil	21·5	19·5
29 „ 30	·1 % Pb	18·0	20·5
31 „ 32	·2 % Pb	16·4	17·7
33 „ 34	·4 % Pb	16·9	16·5

According to this experiment therefore the effect of the lead was not more marked than was the case with the oat crop grown in 1914 immediately after the application of the galena.

4. *Immediate influence of Galena upon Trifolium.*

In order to find whether galena immediately after its application to the soil acts very unfavourably upon trifolium another series of pots was arranged, the soil and admixture with galena being the same as already described for Pots 27–34. The pots were filled May 12th, 1915, and the seed sown the same day The crop was cut August 23rd, and then air-dried and weighed. See Photograph "F." The results are given in Table XIV.

TABLE XIV.

Immediate influence of Galena upon the growth of Trifolium.

No. of pots	Treatment	Average weight of crop (gm.)
53, 54 and 55	Nil	11·8
56 and 57	·1 % Pb	8·7
58 „ 59	·2 % Pb	3·6
60 „ 61	·4 % Pb	·7

5. *Effects of Blende and of Iron Pyrites upon Oats.*

The minerals were finely ground and the manner of procedure was similar to that already described in the experiment with galena. The treatment and yields are given in Table XV.

TABLE XV.

No. of pots	Treatment	Average weight of straw (gm.)	Average weight of grain (gm.)
35, 36 and 37	Untreated	22·5	19·3
38 and 39	Blende = ·05 % Zn	18·5	20·2
40 „ 41	„ = ·1 „ „	15·0	21·2
42 „ 43	„ = ·2 „ „	17·2	21·0
44 „ 45	„ = ·4 „ „	14·7	18·0
23 „ 24	Pyrites ·2 „	19·2	25·2
25 „ 26	„ ·8 „	34·2	32·2

See Photograph "G."

The most important features of the results of this experiment are

(*a*) The reduction in the weight of straw but not of grain in the blende pots.

(*b*) The remarkable increase in crop production due to the application of iron pyrites. This was largely due to improved tillering.

6. *Effects of Blende and of Pyrites upon Trifolium incarnatum.*

Trifolium was taken as a catch crop after the oat crop of Experiment 5. Germination was satisfactory throughout; but gradually the plants perished so that in six weeks time there were none left except in the control pots.

7. A crop of mustard was taken after the trifolium of Experiment 6. The results are given in Table XVI.

Table XVI.

Effects of Blende and Pyrites upon Mustard.

No. of pots	Weight of crop (gm.)
35, 36 and 37	16·1
38 and 39 (·05 % Zn)	6·8
23 and 24 (·2 % FeS_2)	5·2

With ·1, ·2 and ·4 per cent. Zn and with ·8 per cent. pyrites the plants flourished for a time but subsequently perished.

II. Remedial Measures to combat the Influences of Mine Refuse.

1. Two soils *A* and *B* were obtained from fields known to be badly affected.

Soil *A* contained ·48 per cent. Pb, ·30 per cent. Zn.

Soil *B* contained 1·43 per cent. Pb, ·02 per cent. Zn, ·06 per cent. Cu.

Table XVII.

Treatment and weight of Oat Crop with Soil A.

M = complete manure including nitrate, phosphate and potash.

No. of pots	Treatment	Average weight of straw (gm.)	Average weight of grain (gm.)
1 and 2	Untreated	1·3	·38
3 „ 4	M	4·8	3·8
5 „ 6	M + ·2 % CaO	54·8	40·8
7 „ 8	M + ·4 % CaO	59·7	41·1
9 „ 10	M + 4 cwt. per acre Na_2SiO_3	9·4	6·2
11 „ 12	M + 4 cwt. per acre $NaMnO_4$	10·9	8·1

The soil treatments and yield of crop are given in Tables XVII and XVIII.

TABLE XVIII.

Treatment and weight of Oat Crop with Soil B.

No. of pots	Treatment	Average weight of straw (gm.)	Average weight of grain (gm.)
13 and 14	Untreated	·2	·02
15 „ 16	M	1·1	·2
17 18	M + ·3 % CaO	31·1	23·8
19 20	M + ·6 % CaO	22·5	18·6
21 22	M + 4 cwt. per acre Na_2SiO_3	3·2	·9
23 24	M + 2 cwt. per acre $NaMnO_4$	1·8	·3
25 26	M + 4 cwt. per acre $NaMnO_4$	3·4	·8

See Photographs "H," "I" and "J."

It should be noted that the sodium silicate appeared to give very good results with both soils up to the end of the second month of the crop's period of growth. From that time onwards, however, the crop made practically no further progress.

2. Trifolium incarnatum was sown for a catch crop after the oat crop of Experiment 1. Satisfactory crops were obtained in the limed pots; but the plants died off in all the other pots.

3. During the following season (1915) a second crop of oats was grown in the limed pots, soil *B* of Experiments 1 and 2. The yields are shown in Table XIX.

TABLE XIX.

No. of pots	Weight of straw	Weight of grain
15 and 16	·8	·08
17 „ 18	19·7	12·7
19 „ 20	19·0	16·8

See Photograph "K."

4. Field experiments have indicated that affected land needs an application of lime which is above the "lime requirement" as usually determined. A pot experiment was carried out with soil *B* of Experiment 1, which was found to have a "lime requirement" of two tons per acre. The results obtained (*a*) with a crop of oats, and (*b*) with trifolium catch crop are given in Table XX. See also field experiments described below.

5. Many farmers maintain that the application of a relatively small quantity of unaffected soil to affected soils greatly improves the fertility of the latter. An experiment with a crop of oats conducted as indicated in Table XXI did not provide any support to this view.

TABLE XX.

(*a*) Oat crop.

No. of pots	Treatment	Weight of straw	Weight of grain
1, 2 and 46	Untreated	·36	·12
5 and 6	·5 % CaO	15·0	8·0
11 „ 12	·3 % CaO	13·8	6·8
21 „ 22	·2 % CaO	12·3	8·0

See Photograph "L."

(*b*) Trifolium.

No. of pots	Weight of crop
1, 2 and 46	Nil—all plants died off
5 and 6	20·6
11 „ 12	5·2
21 „ 22	Nil

See Photograph "M."

TABLE XXI.

No. of pots	Unaffected soil added	Weight of straw	Weight of grain
47 and 48	Nil	1·0	·35
49 „ 50	2 %	1·0	30
51 „ 52	8 %	1·2	·35

See Photograph "N."

It is possible that where in field practice the application of unaffected soil has been very beneficial, the affected soil has been detrimentally influenced in mechanical composition. Soil *B* used in this pot experiment did not differ much in this respect from the adjoining unaffected soil.

FIELD EXPERIMENTS.

War conditions interfered with facilities for continuing with field experiments which had been commenced. The few results obtained, however, agreed in most respects with those of the pot experiments. But, as regards liming, the field trials indicated very clearly the necessity for a much heavier dressing of lime than that found effective to restore fertility in the pot experiments. (See Pot Experiment II, 4, above.) With a heavy dressing of lime there appears to be a permanent improvement. *Photograph "X" shows the effect of lime persisting at the end of eight years.*

General Conclusions.

1. There is an area of about 3000 acres within the sphere of influence of mine refuse in North Cardiganshire.

2. The unproductiveness of the affected land is due to the simultaneous influences of a number of circumstances, e.g.

(*a*) The presence in the soil of toxic substances, mainly lead and zinc, but in some cases small quantities of copper also. In the neighbourhood of a few mines contamination by iron pyrites and marcasite may be contributory causes.

(*b*) Unfavourable changes in the mechanical composition of the soil leading to deterioration of its physical properties, e.g. capacity to retain water.

3. The deleterious effects are much more pronounced upon some crops than others. Leguminous plants appear to be the most susceptible to the injurious effects.

4. Lead and zinc are carried on to the soil in the form of galena and blende respectively. These sulphides are soon acted upon in the soil and converted into other compounds of lead and zinc. It appears probable that the lead is, in some measure, retained by the humic or other colloidal substances of the soil.

5. Lead is absorbed by and enters into the composition of the plant, but only in very minute quantities. The poisoning of animals is due more to the lead deposited on the exterior than to that contained within the plant.

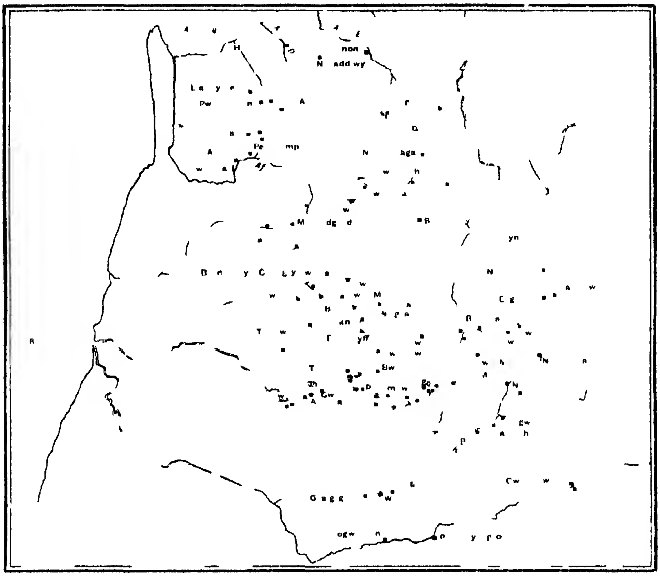
6. The application of sodium silicate to the soil tends to mitigate the undesirable influences of mine refuse upon plant growth. But the most effective remedial measure is the application of a heavy dressing of lime. The amount of lime applied should be considerably greater than the "lime requirement" as determined by the absorption of lime from calcium bicarbonate solution.

7. Apart from liming, it is by preventive rather than remedial measures that the farmer's losses with both land and livestock may best be minimised. These measures should involve

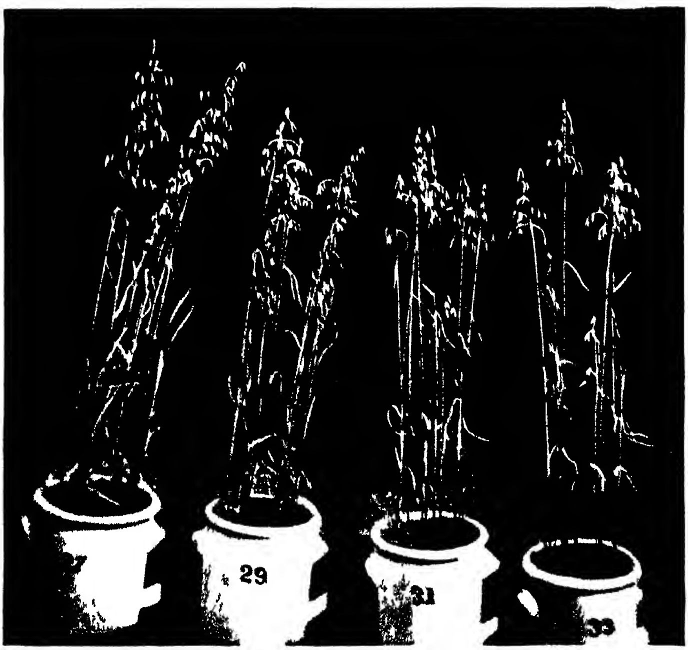
(*a*) careful management at active mines to reduce as far as possible the amount of injurious ingredients allowed to flow into rivers;

(*b*) guarding against pollution from disused levels;

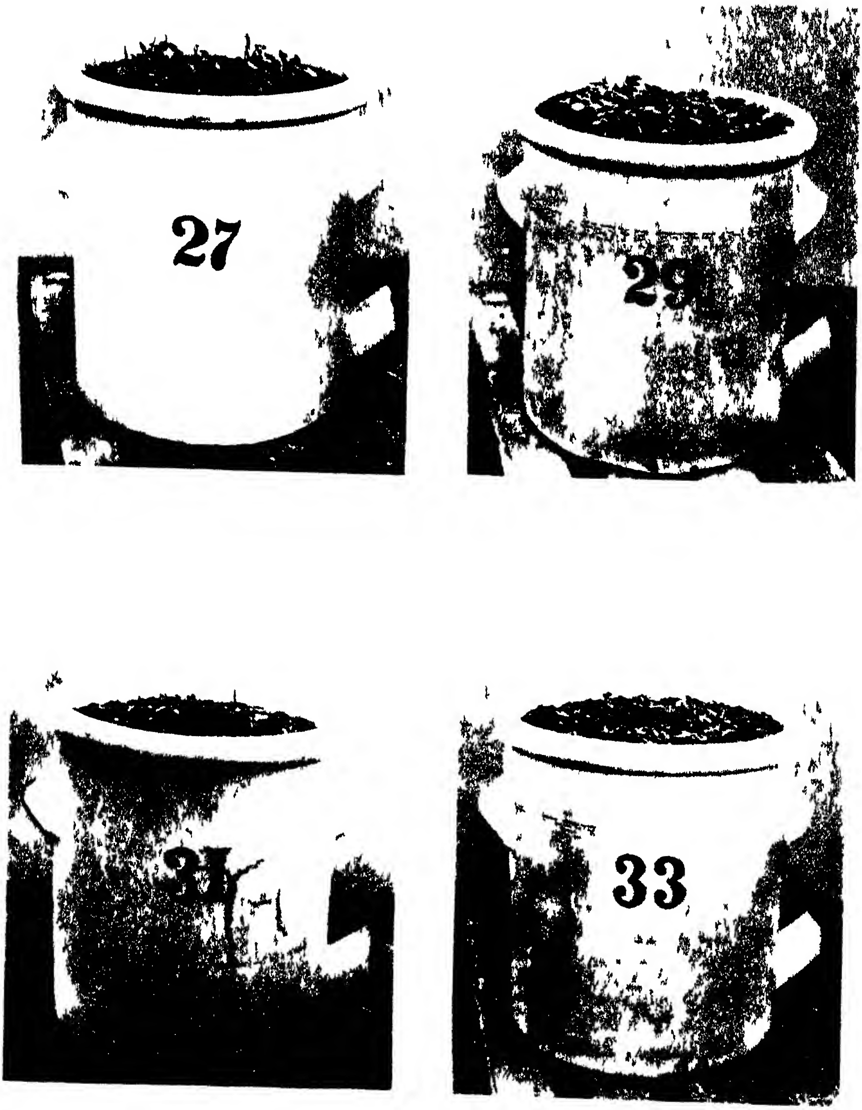
(*c*) protection of mine slime and sand heaps at both active and disused mines so as to prevent the material from being washed into the water courses;



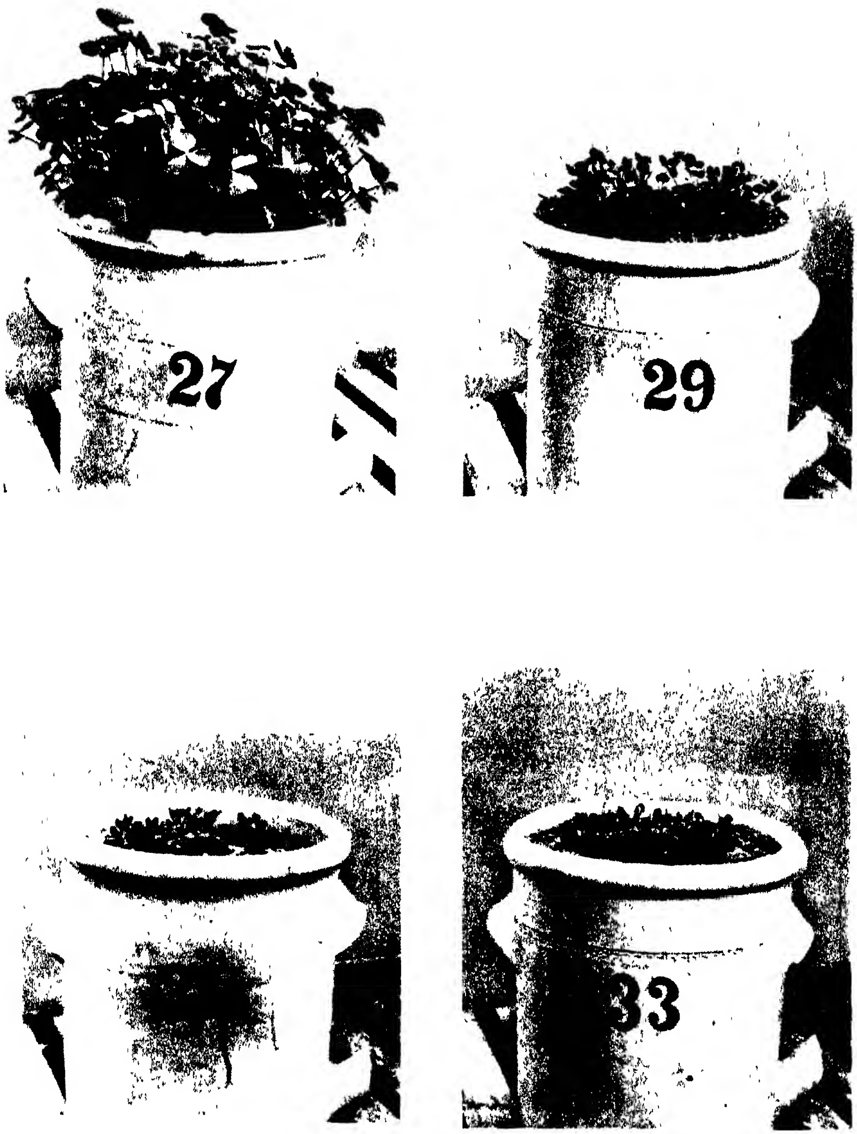
A Map showing Rivers and most important Mines of North Cardiganshire Scale 1 8·6 Miles



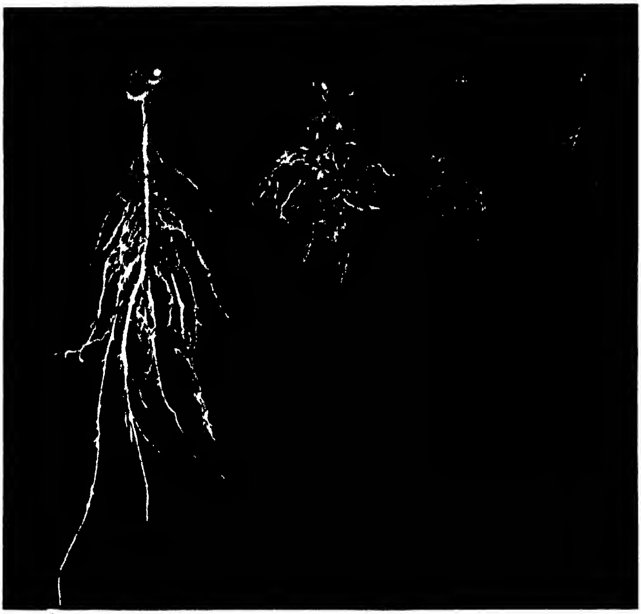
B Effect of galena upon Oats See p 388



C Influence of Galena upon Trifolium catch crop See p 389 Photograph taken January 5th 1915

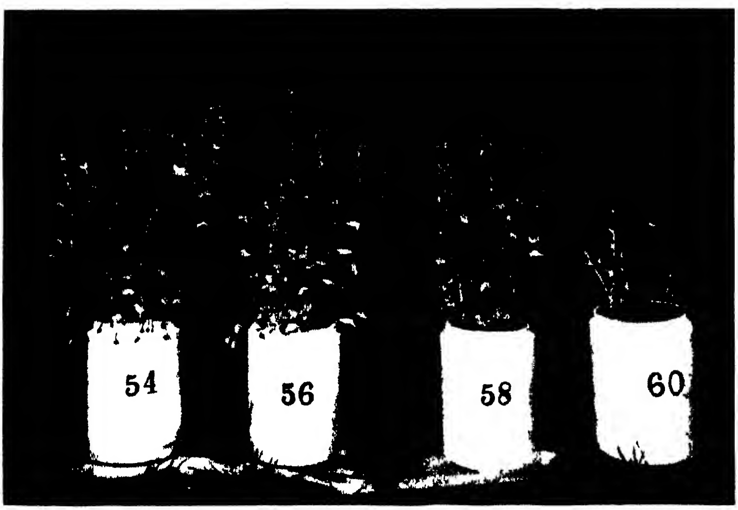


"D." Influence of Galena upon Trifolium catch crop. Photograph taken March 25th, 1915. See p. 389.

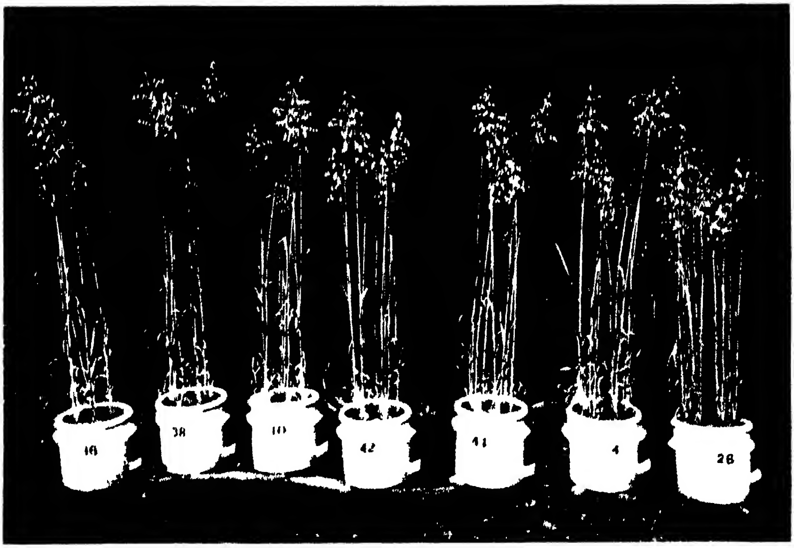


Nil 1 Pb 2 Pb 4 Pb

F Showing effects of Galena upon roots of Trifolium plants See p 389



"F" Showing immediate influence of Galena upon Trifolium See p 390

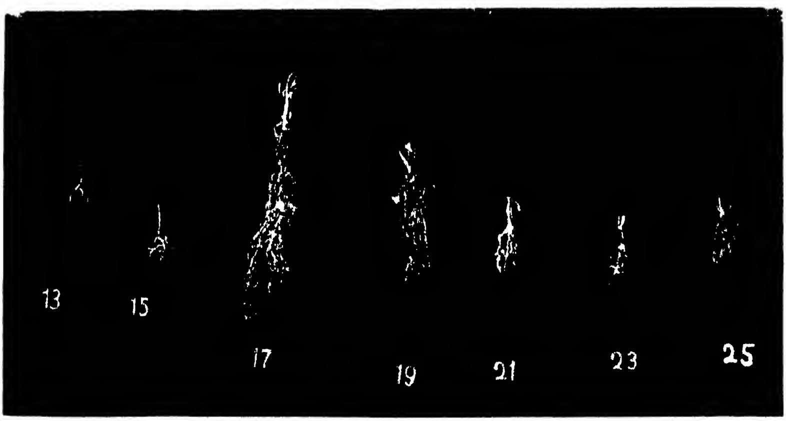


Nil 0·5 Zn 1 Zn 2 Zn 4 Zn 2 FeS 8 FeS$_2$

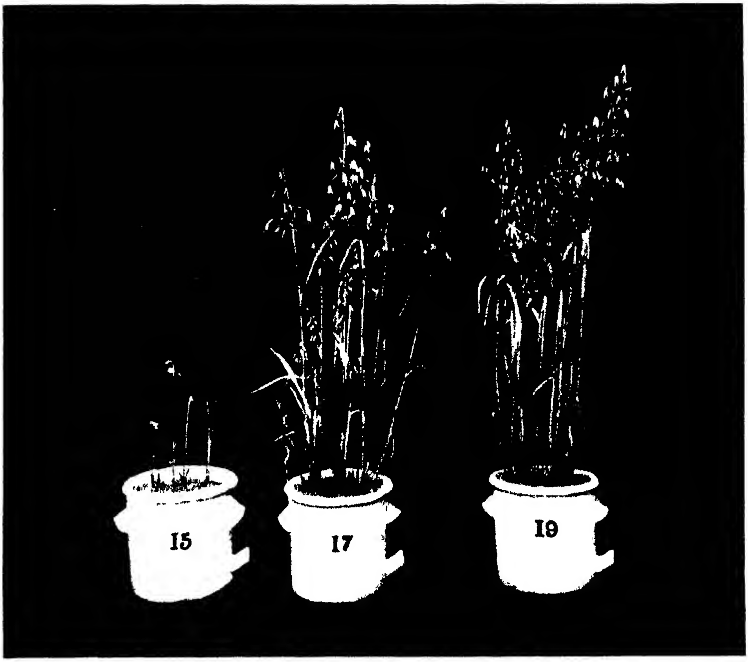
G Effects of Blende and Iron Pyrites upon Oats See p 390

"H" Effects of various treatments of affected soil. See p 391, Table XVII

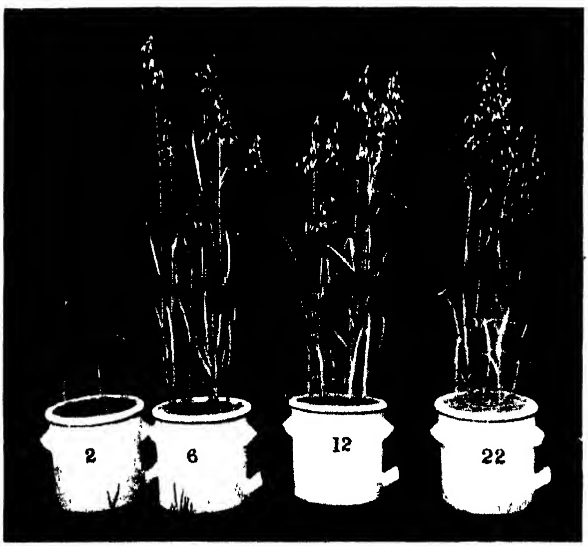
I Showing effects of various treatments of affected soil See p 392 Table XVIII



"J" Roots of Oats from pots shown in Photograph "I



"K." Showing beneficial effects of lime in second year after application. See p 392, Table XIX.

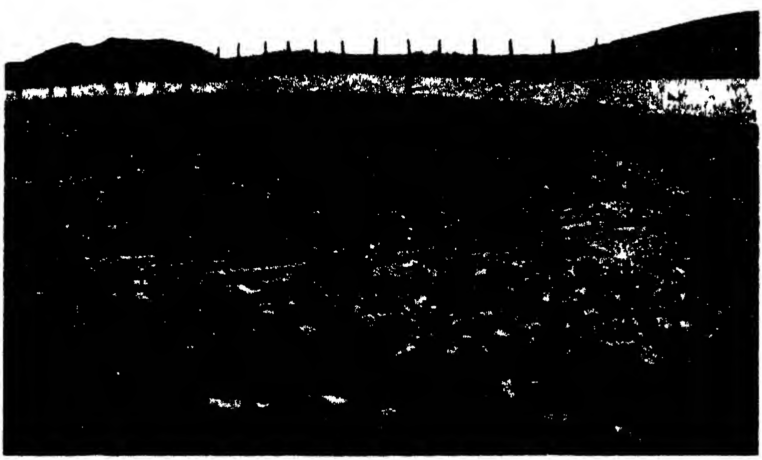


"L." Showing effects of various quantities of lime. See p. 393, Table XX (*a*).

"M Effects of various dressings of lime upon growth of Trifolium See p 393, Table XX (*b*)

"N" Effects of adding fertile soil to affected soil. See p. 393, Table XXI.

"O" Through the agency of the atmosphere in the neighbourhood of lead mines vegetation is destroyed
Subsequently, on the slopes, all the soil may be washed away by rain

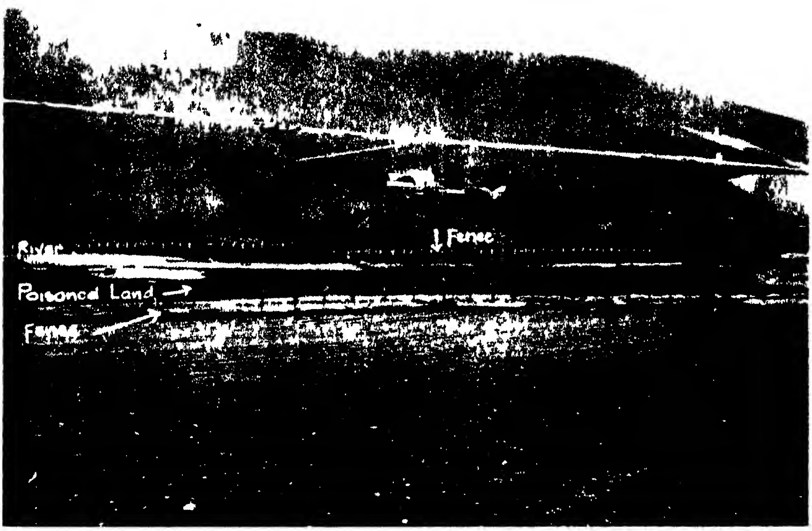


"R Photographs "P," "Q and ' R" show bare patches in turnip field caused by mine water overflowing from leat shown at top of photographs

S

'T Photographs S and 'T show bare patches in Oat field, due to contamination of soil with mine refuse

' U Slime heap (mine refuse) covering several acres of land gradually being washed down to river below and thus continually contaminating land further down the valley



W Many miles of leats constructed to conduct water from one mine to another The water often overflows so that the land lower down the slope is poisoned and made worse than useless

"X" Field poisoned 30 years ago Portion of field limed 8 years ago Whole of field sown with wheat, but crop grows on limed part only See p 393

(*d*) due attention to the construction of river and leat embankments and proper care of existing ones;

(*e*) selection of stock, usually cattle, least susceptible to lead poisoning for grazing affected pastures;

(*f*) giving a large area of unaffected along with the affected land for grazing so as to avoid close confinement to poisoned herbage;

(*g*) allowing the washing of pastures by rain after floods before further grazing;

(*h*) fencing off the very worst of the poisoned land. See Photograph "V";

(*i*) threshing contaminated hay before it is supplied to the animals;

(*j*) the strict avoidance of utilising mine wastes from ditches, etc., for any purpose that may lead to injury to land or stock.

In conclusion I wish to thank Dr E. J. Russell for several valuable suggestions, and also the Commissioner of Agriculture for Wales, C. Bryner Jones, Esquire, for his kind interest in the work.

(*Received May* 10*th*, 1919.)

A NOTE ON THE CAPILLARY RISE OF WATER IN SOILS.

By BERNARD A. KEEN.

(Goldsmiths' Company's Soil Physicist, Rothamsted Experimental Station.)

VERY diverse views are expressed on the height to which water can rise in soils under the forces of capillarity. Alway and MacDole[a] in the course of a brief historical review, point out that these estimates range from two or three feet only, to as much as two or three kilometres, although the majority do not exceed 200 feet. Most of the investigators who advance a high value for the capillary rise are careful to point out that in all probability the movement of water in this case would be exceedingly slow, owing to the excessive friction in the minute capillary spaces. Actual experiments on the rise of water in tubes of compacted soil result in low values, which are in all probability exceeded in the field. Warington in his book *Physical Properties of Soil* gives a typical table showing the results of Loughridge[b] for Californian soils. The figures are reproduced here in Table I. They show, as would be expected, the rapid

TABLE I.

Rise of water in four Californian Soils.

No.	1 hour	6 hours	1 day	2 days	6 days	12 days	26 days	125 days	195 days
	in.	in.	in.	in.	in.	in.	in.	in.	in.
1	8	12½	14	15	16½	—	—	—	—
2	9½	19	27	30½	35	38	41	47	--
3	2	9	13	17	20½	25	31½	41	50
4	1½	6	10½	14½	—	23	26½	--	46

Mechanical Analysis of above soils (Hilgard's method)*.

Soil	Clay	Fino silt	Coarse silt	Fine sand
1. Sandy soil	2·82	3·03	3·49	89·25
2. Alluvial soil	3·21	5·53	15·42	72·05
3. Silty soil	15·02	15·24	25·84	45·41
4. Adobe soil	44·27	25·35	13·47	13·37

* The clay, silt and sand differ in dimensions from the fractions of the same name in British analyses.

[a] *Journ. Agric. Res.* **9** (1917), p. 27.

[b] *Californian Expt. Sta. Rep.* (1892–4), p. 91.

initial rise in the coarser textured soils and the higher final value in the fine textured soils, and it will be seen that the maximum value is only four feet. It is practically impossible to reproduce with certainty, under laboratory conditions, the soil structure as it exists in the field. Leather[a] has pointed out in some remarks on the permeability of soils that it is not difficult to fill a series of cylinders with portions of the same soil so nearly uniformly alike that the rate of flow through each one is approximately the same. But an agreement of duplicates thus obtained is not evidence that the field conditions are reproduced by the laboratory experiments, and these considerations apply equally to laboratory experiments on the capillarity of soils.

It is thus a matter of considerable interest and importance to obtain values for the possible capillary rise in soils. Mitscherlich[b], in the course of an extensive investigation into various physical properties of soil, has effected such a calculation. It is an indirect one, because it involves a relation between the experimentally observed heat of wetting (Benetzungswärme), and the surface of the soil particles. The calculation leads to enormous values for the possible capillary rise—2 or 3 kilometres for heavy clays and loams. His maximum experimental result, however, did not exceed 80 cms. over a three months period.

In the present note is given a simple direct calculation, the results of which may be taken as probable maximum values for the capillary rise. The calculation is based on the following assumption: if the soil grains are taken as spherical, of one size and packed in the closest possible manner, then the pore space may be regarded as consisting of capillary tubes having an approximately triangular cross section. The dimensions of these tubes have been obtained by Slichter[c] who introduced this conception and justified it in the course of an extensive mathematical treatment of the flow of air and water through an "ideal" soil. The mean value of the triangular cross section is $\cdot 2118r^2$ where r is the radius of the soil grain. This triangular pore changes in cross sectional area as it follows the surface of the soil grains, passing alternately through maximum and minimum values. The minimum value is about $\cdot 1475r^2$, which is roughly 30 per cent. less than the average mean value $\cdot 2118r^2$. The present writer agrees with these calculations, and has used the results as shown immediately below.

Consider the height, h, which water would reach in a tube whose

[a] *Journ. Agric. Sci.* **4** (1911–12), p. 304.

[b] *Landw. Jahrb.* **30** (1901), p. 361.

[c] King and Slichter, *19th Ann. Rep. U.S. Geol. Survey* (1899), pt 2.

cross section is an *equilateral* triangle of side K, assuming that the tube is wetted by the water so that the angle of contact is zero.

A simple calculation[a], ignoring small corrections, gives the value as:

$$h = \frac{4\sqrt{3}T}{\rho g K} \qquad \text{.........(1)},$$

where T = surface tension,

ρ = density of the water,

g = force of gravity,

and h, K have the meanings assigned above.

The cross sectional area of the tube is

$$\frac{\sqrt{3}K^2}{4},$$

and no considerable error will be made by putting this equal to the mean value $\cdot 2118r^2$ deduced by Slichter for the soil-pore.

Thus

$$\frac{\sqrt{3}}{4}.K^2 = \cdot 2118r^2 \qquad \text{.........(2)},$$

whence $K = \cdot 7r$ very nearly.

Substituting the value of K in (1):

$$h = \frac{4\sqrt{3}T}{\cdot 7\rho g r} = \frac{9\cdot 9T}{\rho g r} \qquad \text{.........(3)}.$$

It is known that the effect of most dissolved salts is to increase the surface tension of water. In the present case, however, the soil is assumed saturated and the soil solution therefore very dilute. We can thus employ the usual value 75 dynes cm.^{2} with very little error.

Taking $\rho = 1$ and $g = 981$ we obtain a value for h:

$$h = \frac{\cdot 75}{r} \qquad \text{.........(4)}.$$

We can substitute various values for r, the radius of the soil grain, in this equation and obtain the corresponding capillary rise, h. It is instructive to take for this purpose the various grain sizes as obtained by the usual mechanical analysis. The results are given in Table II.

It will be seen that the possible height of capillary rise rapidly increases as the diameter of the grains diminishes. Equation (4) from which

[a] See for instance Poynting and Thomson, *Properties of Matter*, 4th ed. 1907, p. 141, where the calculation is given for a circular tube. Exactly the same principle holds for a triangular tube.

the values are calculated gives in fact a rectangular hyperbola for the curve connecting h and r, and as r becomes very small, the value of h increases exceedingly rapidly. The values for the various grain sizes given in Table II refer, as already stated, to an "ideal" soil in which the grains are all of one size, spherical and packed in the closest possible manner.

TABLE II.

Soil fraction	Diameter in mm. max.	Diameter in mm. min.	Capillary rise in cms. min.	Capillary rise in cms. max.	Average rise in feet
Fine gravel	3	1	5	15	⅓
Coarse sand	1	·2	15	75	1½
Fine sand	·200	·040	75	375	7½
Silt	·040	·010	375	1500	31½
Fine silt	·010	·002	1500	7500	150
Clay	·002	—	7500	—	150 upwards

It is quite certain that these figures must be considerably reduced for actual soils made up of a mixture of particles of all shapes and sizes in which the capillary spaces are irregular in length, width and also direction. The irregular shape of the grains will result in a narrow capillary tube suffering a considerable enlargement of its effective cross section at some point, and this will greatly reduce the amount of the possible capillary rise. The trapping of air in the interstices between the grains will also act in the same direction. Finally in the heavier types of soil containing much clay, the swelling of the colloidal portion due to inbibition of water will completely close some of the openings and will reduce the diameter of others to such extent that the movement of water through them will be extremely slow.

The values in Table II for various soil fractions may therefore be regarded as the extreme limits of capillary rise for actual soils of corresponding average pore area. In all probability these values are considerably in excess of those occurring in practice. On the other hand, laboratory experiments give minimum values for the capillary rise, because it is impossible to reproduce, as in the field, the long continued oscillation of meteorological and soil water variants; which results in the progressive compacting of the surface soil and subsoil into the most favourable position for the production of the capillary effects.

(*Received July 3rd,* 1919.)

A QUANTITATIVE RELATION BETWEEN SOIL AND THE SOIL SOLUTION BROUGHT OUT BY FREEZING-POINT DETERMINATIONS.

By BERNARD A. KEEN.

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Introduction.

An interesting series of papers has recently been published by Bouyoucos[1] and his fellow workers, on the nature and composition of the soil solution. The results appear to be of considerable significance, pointing to the existence of intimate and complex relationships between the soil and its water content, and possible qualitative explanations of the observed facts are advanced by the authors. The present paper is an attempt to give a definite quantitative expression to some of these relations, by means of a critical examination of part of the experimental data recorded in the papers mentioned. Bouyoucos points out that the various methods hitherto employed to study the soil solution give data which at the best are scarcely qualitative as to the actual concentration in the soil complex; they entirely fail to give any information on the physical relationships existing between soil and its water content. Using other methods of attacking the problem he is able to carry it a stage nearer solution. His results show broadly that the water in soil behaves differently from that in sand, in that it exists in two different conditions, called by him "free" and "unfree." This terminology is employed throughout the present paper, for convenience in referring to Bouyoucos' results, and because we have not, at present, sufficient information to justify the immediate use of more definite terms. The names are not, however, entirely satisfactory. It is quite possible that some of the water in the "unfree" state may be capable of evaporating directly from this condition when the soil is drying, and on the other hand, it is by no means certain that all the "free" water is really free in the strict sense of the word.

[1] Michigan Agric. Coll. Expt. Station. *Tech. Bulls.* Nos. **24** (1915), **31** (1916), **36** (1917), **37** (1917), **42** (1918).
Also in *Journ. Agric. Res.* **8** (1917), p. 195; **15** (1918), p. 331.

The present writer has shown[1] that the course of the evaporation of water from the soil can only be explained on the assumption that an intimate connection exists between the soil and its moisture content over a wide range. The evaporation was quite different from that shown by moist sand. The latter could be readily explained from known laws of diffusion, but in the evaporation from soil other factors were distinguished. A definite mathematical expression was found for this

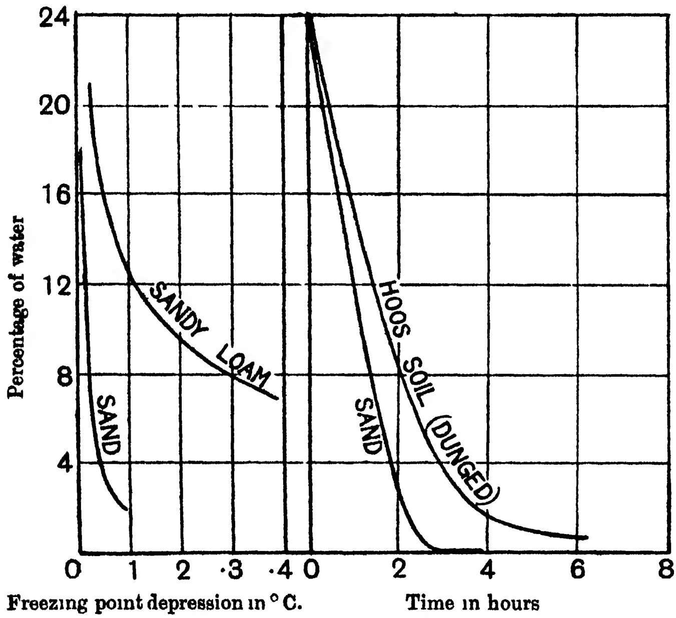

Fig. 1. Comparison for sand and soil at varying moisture contents, of freezing-point depression and evaporation.

relationship, and all the available data pointed to the importance of the soil colloids in controlling it. These differences in the relations of sand and soil to their water content can be very well seen by comparing the writer's curves showing the evaporation from sand and soil, with Bouyoucos' curves for the depression of the freezing-point at various moisture contents (Fig. 1). It is significant that two such diverse methods of examining the soil solution should both show a fundamental difference in its relation to a group of inert particles such as quartz sand on the one hand, and to soil on the other.

Bearing these facts in mind one can readily understand the incomplete and sometimes conflicting results obtained from all methods of

[1] B. A. Keen. *Journ. Agric. Sci.* 6 (1914), p. 456.

investigating the soil solution which depend on its isolation from the soil[1].

None of these methods can give, as a final product, the soil solution in the state in which it exists in the soil. Only a fraction of the total moisture content can be obtained by direct methods such as centrifuging, while indirect methods such as mixing the soil with water and filtering, give a solution bearing an unknown, but probably qualitative, relation to the original soil solution. In other words, any modification of the moisture content causes a change in the complicated colloidal phenomena existing in the soil, and hence the portion of soil solution extracted will bear no simple relation to either the portion unextracted, or the original amount.

Further progress in our knowledge of the soil solution must depend on the use of fresh methods. Bouyoucos has attacked the problem in two ways, of which brief descriptions follow. The essential feature is that in each case the solution is examined *in situ*.

Dilatometer and Freezing-point Methods of Investigating the Soil Solution.

The dilatometer method is an application to soil of Foote and Saxton's[2] experiments on the freezing of inorganic hydrogels. The moist soil is placed in the bulb of the dialatometer and the free space then filled with ligroin. From the reading of the meniscus and the known bore of the tube, the expansion occurring when some of the soil moisture freezes can be calculated, and hence the amount of water frozen. It was found that the water present in soil did not all freeze at one given temperature (– 1·5° C.) and the amount which failed to freeze varied considerably in different soils. A similar result was obtained at – 4° C. and – 78° C., although the amounts of unfrozen water were smaller, especially in the colloidal types. No definite relationships could be traced between the amounts of unfrozen water at these temperatures.

On the basis of these results, the soil temperature is classified into three groups: "free," freezing at – 1·5° C.; "capillary-adsorbed," freezing at – 4° C. down to – 78° C.; and "combined," not frozen at – 78° C. The divisions of course merge insensibly into one another, but the values obtained at the temperatures chosen are considered as giving

[1] A summary of these methods is given by Bouyoucos (*Tech. Bull.* No. **24**), and also by Stiles and Jørensen (*Journ. Ecology*, **2** (1914), p. 245). The latter is the more detailed account, but does not include the Morgan oil pressure method (*Soil Sci.* **3** (1917), p. 531).

[2] *Journ. American Chem. Soc.* **38** (1916), p. 588; **39** (1917), p. 1103.

very fair approximations to the amounts present in these three forms. The actual amount of any one division varies considerably from soil to soil, but generally speaking, the amount of free water decreases, while the capillary-adsorbed and combined water increase as the soils pass from non-colloidal to colloidal in type, although Bouyoucos finds many exceptions to this rule.

It will be observed that the dilatometer method as used gives qualitative information only. The observed fact that the capillary-adsorbed group passes gradually at one end to combined water, and at the other to free, suggests that the variants controlling its relation to the soil alter in a continuous manner over the whole range of this division, a view confirmed by the present writer's experiments cited above. This view is also supported by Bouyoucos' second set of experiments on the lowering of the freezing-point of the soil solution.

These experiments were done in the usual Beckmann apparatus, on the moist soils and sands. It was found that solidification could be readily induced, except when the moisture content was quite low. In quartz sand for instance, the lowering of the freezing-point could be measured when the moisture content was only 0·7 per cent.

In quartz sand and some extreme types of sandy soil, the depression of the freezing-point was found to be approximately inversely proportional to the moisture content, *i.e.*

$$MD = K, \quad . \quad . \quad . \quad . \qquad (1)$$

where M = moisture content,
D = depression of freezing-point,
K = constant.

With soils a different relationship holds, the depression of the freezing-point increasing in geometrical progression as the moisture content decreases in arithmetical progression. Bouyoucos interpreted this as indicating that the soil solution increases in concentration at a greater rate than would be accounted for by the known decrease in total moisture content, and the general hypothesis is advanced that in soils some of the water is rendered "unfree," and thus does not enter into the actual soil solution, as determined by the freezing-point method. It is the quantitative examination of this suggestion, which is supported by a considerable amount of evidence in addition to that advanced by Bouyoucos, with which the present paper is mainly concerned.

TYPICAL EXPERIMENTAL DATA GIVEN BY BOUYOUCOS.

For convenience, typical data for quartz sand and four types of soil are reproduced here, in Tables I and II, and Fig. 2.

TABLE I.

Lowering of the Freezing-point of Quartz Sand at various moisture contents.

Percentage of moisture	Observed lowering of the freezing-point ° C.	Constant (K)
2	·091	·182
6	·027	·162
10	·018	·180
14	·012	·168
18	·009	·162

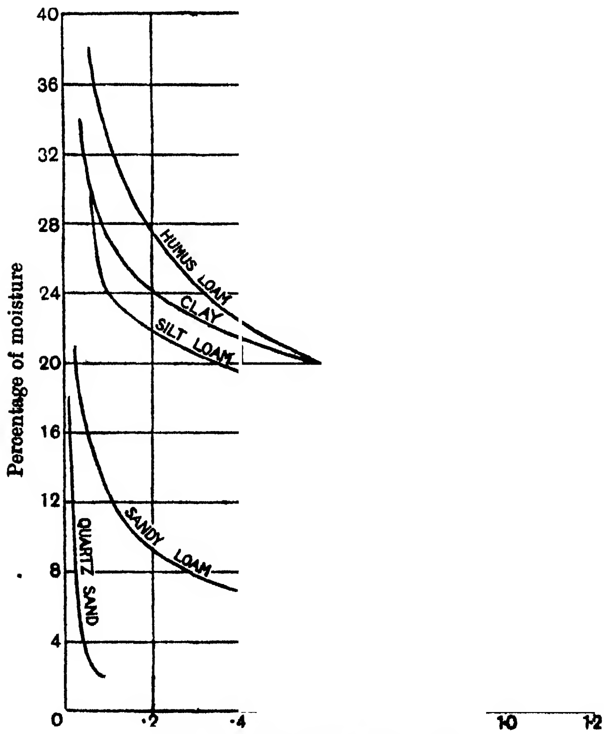

Fig. 2. Increase in freezing-point depression, with decrease in moisture content (Bouyoucos).

TABLE II.

Lowering of the Freezing-point of various types of Soil at different moisture contents.

Clay: Percentage of moisture	Clay: Observed lowering of freezing-point	Clay: Calculated lowering of freezing-point	Silt Loam: Percentage of moisture	Silt Loam: Observed lowering of freezing-point	Silt Loam: Calculated lowering of freezing-point
18	·922	—	16	·860	—
20	·580	·6242	18	·560	·688
22	·307	·3916	20	·350	·448
24	·212	·2078	22	·200	·280
26	·127	·1435	24	·095	·160
28	·082	·0859	26	·076	·076
30	·062	·0555	28	·071	·0608
32	·042	·0419	30	·060	·0568
34	·034	·0284	—	—	—
Humus Loam			**Sandy Loam**		
14	1·200	—	7	·390	—
18	·760	·7888	9	·220	·2925
22	·420	·4995	11	·130	·1650
26	·245	·2761	13	·087	·0975
30	·140	·1610	15	·065	·0642
34	·089	·0920	17	·040	·0487
38	·0585	·0585	19	·030	·0300
—	—	—	21	·025	·0225

Discussion of the Experimental Data.

Taking the values for quartz sand (Table I) it is seen that very fair agreement holds with equation (1) above, the freezing-point depression being inversely proportional to the moisture content. This points to the moisture in the sand obeying the same law as dilute solutions—the freezing-point depression varying approximately as the concentration.

With soils this relation does not hold, but is replaced by an approximate geometrical progression ratio. The question at once arises, is any proportion of the soil moisture "unfree," in the sense that it is not part of the soil solution as understood in these experiments?

If this were so the soil solution would become increasingly concentrated as the total moisture content decreased towards the value of this unfree water. To repeat the example given by Bouyoucos in support of this idea, a clay soil which renders 15 per cent. of water unfree, by adsorption or chemical combination for instance, and which at 36 per cent. and 18 per cent. of total moisture content gave depressions of ·034° C. and ·955° C. respectively, would have available for the soil

solution, 21 per cent. and 3 per cent. of water in the two cases. Hence one would expect the freezing-point lowering to be many times greater in the second case, than in the first. This idea can be easily tested:

Let Z be the water rendered unfree. Then if the inverse proportionality law still holds for the remaining ("free") water we have:

$$D(M - Z) = K, \qquad (2)$$

where M = total moisture content,
D = corresponding depression,
K = constant.

This equation should define the soil curves, *i.e.* the curves would still be rectangular hyperbolae, of the type given by the equation $MD = K$ for quartz sand, with the D axis displaced a distance Z along the M axis. Substituting in equation (2) for the sandy loam soil (Table II) at moisture contents of 7 per cent. and 21 per cent.:

$$\cdot390\,(7 - Z) = K,$$
$$\cdot025\,(21 - Z) = K.$$

Solving these two equations we have, very approximately,

$$Z = 6; \quad K = \cdot390;$$

and hence the general equation becomes

$$D(M - 6) = K = \cdot390. \qquad (3)$$

A test of this equation for the remaining values of M and D, leads to the following values for K:

TABLE III.

M	K
7	·390
9	·660
11	·650
13	·609
15	·585
17	·440
19	·390
21	·390

There is a considerable change in K, hence the assumption that a definite amount of water is rendered unfree by the soil is incorrect, if the inverse proportionality law for M and D is considered to hold. This result leads to two alternatives, either the law does not hold, which means that the effective proportion of dissolved salts alters with moisture content, or else the actual amount of unfree water changes with total

moisture content. It is of course possible that both of these alternatives will operate together in the soil. The former will almost certainly occur, to judge by a general survey of the data Bouyoucos advances for the concentration of the soil solution. Its explanation is a matter of great complexity and is not considered in the present paper, which is devoted to the second alternative, that the actual amount of unfree water changes with the total moisture content.

Let Y_n be the amount of *free* water, when the total moisture content is M_n, and D_n be the corresponding freezing-point depression. We have the general equation:

$$Y_n D_n = K, \text{ where } K \text{ is a constant,}$$

or

$$Y_n = \frac{K}{D_n}.$$

Using again the figures of D_n for the sandy loam soil (Table II) we obtain a series of values for Y_n:

$$Y_7 = \frac{K}{\cdot 390}; \quad Y_9 = \frac{K}{\cdot 220} \dots Y_{19} = \frac{K}{\cdot 030}; \quad Y_{21} = \frac{K}{\cdot 025}.$$

The suffix of Y indicates the corresponding value of M_n, the total moisture content. The values of Y_7 to Y_{19} can be obtained in terms of Y_{21}, by substituting $\cdot 025\, Y_{21}$ for K in each case:

$$\left.\begin{aligned} Y_{19} &= \cdot 833\ Y_{21} \\ Y_{17} &= \cdot 625\ Y_{21} \\ Y_{15} &= \cdot 385\ Y_{21} \\ Y_{13} &= \cdot 287\ Y_{21} \\ Y_{11} &= \cdot 192\ Y_{21} \\ Y_{9} &= \cdot 114\ Y_{21} \\ Y_{7} &= \cdot 064\ Y_{21} \end{aligned}\right\} \quad . \quad . \quad . \quad . \quad (4)$$

This set of equations shows that the actual amount of free water rapidly diminishes as the total moisture content decreases. For instance, comparing Y_{19} with Y_9, the amount of free water decreases to about $\frac{1}{7}$th, while the total water is decreased only by $\frac{1}{2}$. This indicates that the amounts of free and unfree water bear respectively a decreasing and increasing percentage ratio to the amount of total water, as the latter diminishes. That the relation of the free, and therefore the unfree water, to the total moisture is quantitative can be clearly seen by an inspection of Fig. 3, in which the amount of free water, expressed in terms of Y_{21} (equations (4)) is plotted against the total moisture. In the same figure the curve for clay soil (Table II) is also given, the values

for Y_n being obtained in exactly the same way as for the sandy loam soil.

The curves both seem to be of the type

$$Y_n = cM_n^x, \quad . \quad . \quad . \quad . \quad (5)$$

where c and x are constant for any one curve, and M_n is total moisture.

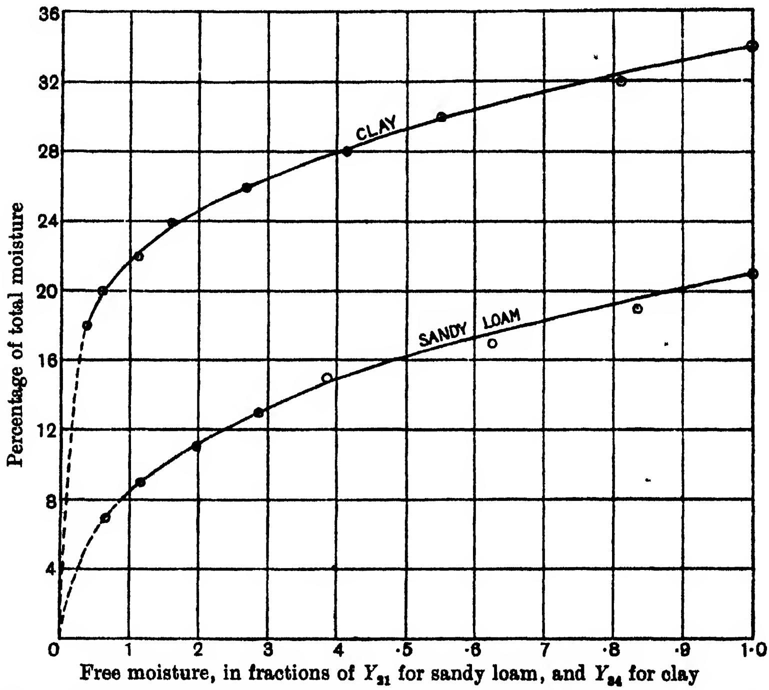

Fig. 3. Relation between free water and total moisture for clay and sandy loam soils.

To test this, a more convenient form of (5) is obtained by taking logarithms of each side:

$$\log Y_n = \log c + x \log M_n, \quad . \quad . \quad . \quad (6)$$

but $Y_n = f \,.\, Y_{21}$, where f varies from 0 to 1. Hence equation (6) becomes

$$\log f + \log Y_{21} = \log c + x \log M_n. \quad . \quad . \quad (7)$$

To solve (7) for c and x convenient values of f and M_n to take from the curve for sandy loam, are

$$(f = \cdot 1,\ M_n = 8\cdot 5); \quad (f = 1\cdot 0,\ M_n = 21).$$

This leads to the values

$$x = 2\cdot 55; \quad \log c = (\log Y_{21} - 3\cdot 366).$$

$$\therefore c = 10^{-3\cdot 366}\, Y_{21}.$$

The values for the clay soil are, $x = 5{\cdot}18$, $c = Y_{34} \,.\, 10^{-7{\cdot}934}$. Hence equation (5) becomes in the two cases,

$$Y_n = 10^{-3{\cdot}366}\, Y_{21} \,.\, M_n^{2{\cdot}55}, \quad . \quad . \quad . \qquad (8)$$

$$Y_n = 10^{-7{\cdot}934}\, Y_{34} \,.\, M_n^{5{\cdot}18}. \quad . \quad . \quad . \qquad (9)$$

To test how accurately these equations fit the curve, it is simpler to return to the logarithmic form, equation (7). This can be written:

$$\log Y_{21 \text{ or } 34} - \log c = x \log M_n - \log f. \quad . \qquad (10)$$

The left hand side of this equation is constant, hence the right hand side should be constant also. In Table IV, the values of $(x \log M_n - \log f)$ are given for various values of M_n and f, taken from the curves. It will be seen that excellent agreement holds over the whole range for both soils. Hence the relation between free and total moisture content is defined by the general equation (5), on the assumption that the freezing-point depression is proportional to the concentration of the soil solution.

TABLE IV.

SANDY LOAM SOIL.

M_n	f	$x \log M_n - \log f$
8·5	·1	3·37
11·2	·2	3·37
13·2	·3	3·38
16·0	·5	3·37
18·65	·75	3·36
21·0	1·0	3·37

CLAY SOIL.

M_n	f	$x \log M_n - \log f$
18	·037	7·934
20	·059	7·969
23	·140	7·906
26	·268	7·901
30	·575	7·891
34	1·000	7·933

If the approximate truth of this assumption be admitted, then we have an important and very interesting insight into the general relations existing between the soil and its moisture content. Of the total moisture present at any time a certain part remains free, and the remainder becomes unfree. The free water is related to the total moisture, and therefore to the unfree water, by a definite mathematical relation over the complete experimental range. We need not at present attempt to define the exact meaning of "free" and "unfree"; indeed there is not, as yet, nearly enough experimental evidence to enable definitions to be made with any degree of accuracy. But it is difficult to resist the con-

clusion that the soil colloids are mainly operative in causing the division, especially when it is noted the general equation (5) connecting free and total moisture is of the familiar adsorption type. The deduction can also be made that as the total moisture content varies, the relative amounts of "free" and "unfree" water also change.

We can obtain a clearer view of the conditions imposed on the soil solution by this equation, if the unfree water (Z_n) is considered.

We have

$$M_n = Y_n + Z_n \quad . \quad . \quad . \quad . \quad (11)$$

and

$$Y_n = cM_n^x. \quad . \quad . \quad . \quad . \quad (5)$$

Equation (5) can be written

$$\frac{1}{c^{\frac{1}{x}}} \cdot Y_n^{\frac{1}{x}} = Y_n + Z_n, \quad . \quad . \quad . \quad . \quad (12)$$

$$\therefore Z_n = \frac{1}{c^{\frac{1}{x}}} Y_n^{\frac{1}{x}} = Y_n. \quad . \quad . \quad . \quad (13)$$

This equation connects the value of the free and unfree water at any moisture content, but owing to the presence of the unknown quantity, Y_{21} (or Y_{34}) in the term $\frac{1}{c^{\frac{1}{x}}}$, the values of Z_n will also contain this same unknown. But we can proceed to obtain a series of values for Z_n in a different manner, using Fig. 3. If we give Y_{21} any arbitrary value, with the obvious restriction that it must lie between 0 and 21, we get a set of values for Y_n at various known total moisture contents, and hence obtain by subtraction the corresponding values of Z_n. Table V shows the values for Y_n and Z_n for the sandy loam soil obtained in this manner, for various assumed values of Y_{21} between 0 and 21.

TABLE V.

Values of Z_n and Y_n for various values of Y_{21}.

	$Y_{21}=21$		$Y_{21}=15$		$Y_{21}=9$		$Y_{21}=5$	
M_n	Y_n	Z_n	Y_n	Z_n	Y_n	Z_n	Y_n	Z_n
21	21·0	0·0	15·0	6·0	9·0	12·0	5·0	16·0
19	16·5	2·5	11·8	7·2	7·0	12·0	3·9	15·1
17	12·4	4·6	8·9	8·1	5·3	11·7	2·9	14·1
15	8·8	6·2	6·3	8·7	3·8	11·2	2·1	12·9
13	6·0	7·0	4·3	8·7	2·6	10·4	1·4	11·6
11	4·0	7·0	2·9	8·1	1·7	9·3	1·0	10·0
9	2·4	6·6	1·7	7·3	1·0	8·0	0·6	8·4
7	1·3	5·7	1·0	6·0	0·6	6·4	0·3	6·7

At first sight the figures appear to show a remarkable relation. If Y_{21} has any value greater than about 9, the actual amount of unfree water increases and then decreases, while the free water continually decreases. It is not until Y_{21} is less than 9 that the free and unfree water both decrease together. The results are shown graphically in Fig. 4, where Y_n is plotted against Z_n[1]. It is necessary to understand that the same *general* equation (13) holds for each curve in Fig. 3. The actual changes in the *numerical* values of Z_n, mentioned above, are due to the fact that Y_{21}, and therefore $\frac{1}{c^{\frac{1}{r}}}$, have a different value for each curve[2].

It is clear from Fig. 4 that if the upper curves were produced they too would show a maximum value for Z_n, but it would eventually correspond to a total moisture content of a greater amount than the undisturbed soil could take up, and is therefore not compatible with the actual conditions. Obviously if Y_{21} were known the true curve showing Z_n and Y_n would also be expressible by equation (13). The question then arises as to which one of the family of curves shown in Fig. 4 best represents the true amounts of free and unfree water in the soil solution, at varying total moisture contents.

We obtain no help in this respect from a knowledge of the fact pointed out above (p. 407) that the percentage ratio of unfree water to total moisture increases as the latter diminishes, because, on testing, it will be seen that each series of values of Z_n in Table V fulfils this condition. It would seem probable that Y_{21} must be fairly large, in view of the low freezing-point depression at that point, but it is not easy to

[1] Actually, Fig. 3 represents a family of parabolae passing through the origin, with their axes inclined to the axes of co-ordinates.

[2] A numerical example may make this clearer. Take as a special case of equation (13):

$$Z_n = A Y_n^{\frac{1}{2}} - Y_n,$$

and let A, the constant, have the values 100 and 5. By simple calculation we then have, in the two cases:

	$A=100$	$A=5$
Y_n	Z_n	Z_n
25	475	0
16	384	4
9	291	6
4	196	6
1	99	4
0·25	49·75	2·25

This table shows that if $A = 100$, Z_n decreases with Y_n, while if $A = 5$ the value increases and then decreases.

conceive of any physical reason why the amount of unfree moisture actually increases and then decreases as the total moisture content diminishes. For this reason it would seem more probable that the real relation is expressed by a curve of the shape given when Y_{21} is put equal to 9, in which case, both Y_n and Z_n decrease in actual numerical value as the soil gets drier, while the percentage ratio they bear to the total moisture content decreases and increases respectively. An

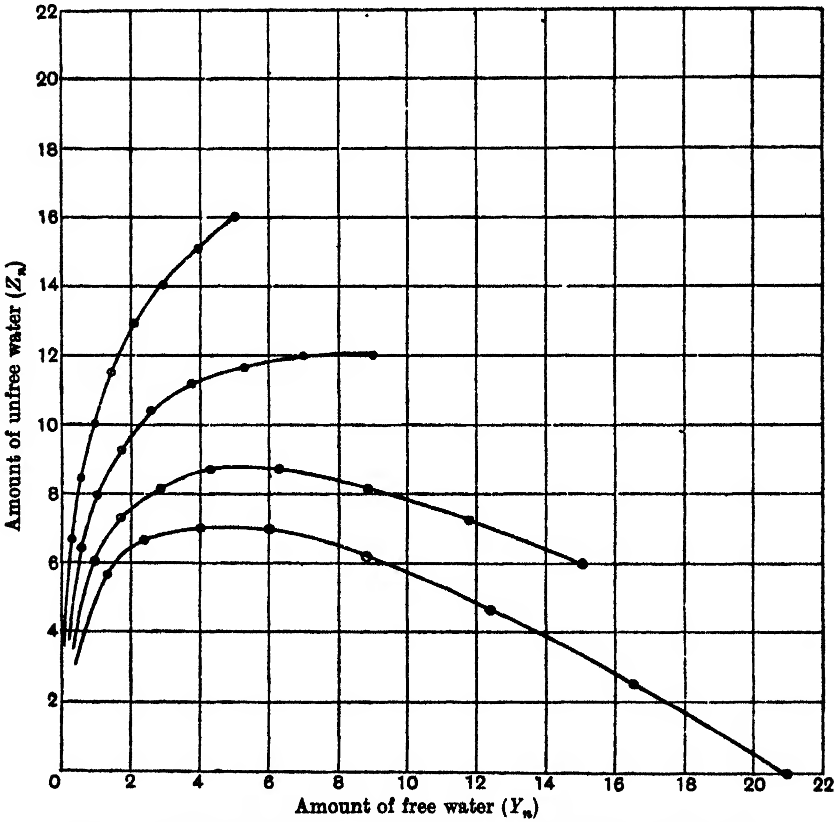

Fig. 4. Relation between free and unfree water, for various values of Y_{21}.

examination of the numerical values for this curve shows that at low percentages nearly all the water is present in the unfree condition. At a total moisture content of 7, only ·58 is free and 6·48 is rendered unfree. The question as to whether this very small amount of free water would make it impossible for the freezing-point depression to be measured is partially answered by the experiments with quartz sand where a moisture content of ·7 per cent. gave satisfactory measurements.

Although it seems more probable that the actual curve is similar to that given in Fig. 4 when $Y_n = 9$, yet if it be assumed that one of the lower curves is nearer the truth, there will be a certain amount of total water, for which the amount of unfree water is a maximum. It is suggestive to associate this point with the "optimum water content" of the soil, at which point, according to Cameron and Gallagher[1], various physical properties of the soil, such as specific gravity, resistance to penetration, rate of warming, etc., reach either a maximum or a minimum value.

This discussion as to the most probable type of curve expressing the relation between free and unfree moisture is put forward with reserve, owing to the very general nature of the possibilities considered. The essential point of the foregoing is that one equation defines the moisture over the whole range, and hence these various constants and critical points represent equilibrium points only, and do not indicate breaks in the physical state of the water in the soil, a conclusion in harmony with that advanced by the present writer in connection with the evaporation of water from soil.

Many other possibilities as to the behaviour of the soil solution could be considered in the light of the present results, but the discussion would necessarily be of a broad nature, and depend on the approximate truth of the initial assumption made in this paper—that the freezing-point depression is inversely proportional to the free moisture content. Hence it does not seem profitable to enter into this aspect of the question, until further information is obtained on this and allied assumptions. Sufficient data have been presented, however, to show that the freezing-point method of examining the soil solution demonstrates that the relations between soil and the moisture content are of no simple type, but that a complex connection holds in a continuous manner over a wide range of water content.

SUMMARY.

An examination has been made of some of the extensive experimental data obtained by Bouyoucos and his associates on the freezing-point depression of soil solution at varying moisture contents, examined *in situ*.

These workers find that the soil solution in quartz sand and extreme types of sandy soil obeys approximately the same law as dilute solutions—

[1] U.S. Bureau of Soils. *Bull.* No. 50 (1908).

the freezing-point depression varying as the concentration, or in the present case, inversely as the moisture content. In other words

$$M_n D_n = K,$$

where K is a constant, and D_n is the freezing-point depression, at a moisture content of M_n. Soils do not obey this law, the freezing-point depression rapidly increasing as the moisture content decreases.

Bouyoucos qualitatively reconciles this difference in behaviour by the assumption that some of the water is rendered unfree, in the sense that it does not take part in the depression of the freezing-point.

The hypothesis is quantitatively examined in the present paper, and assuming its truth, it has been shown that:

(1) The water rendered unfree is not a constant amount, but varies with the total moisture content;

(2) A definite relation exists between the free, unfree and total moisture, expressed by the equations:

$$Y_n = cM_n,$$

$$Z_n = \frac{1}{c^{\frac{1}{x}}} Y_n^{\frac{1}{x}} - Y_n,$$

where c and x are constants for any one soil,

$$M_n = \text{total moisture content},$$
$$Y_n = \text{free water},$$
$$Z_n = \text{unfree water}.$$

(3) The proportion of free to total water continually decreases and that of unfree to total continually increases as the total moisture diminishes in amount, over the experimental range.

(4) The actual amount of free water continually decreases as the total moisture diminishes, but it cannot be definitely stated at present how the actual amount of unfree water changes as total moisture diminishes. The uncertainty is due to the presence of an unknown (but constant) factor in the constant "c" of the equation, viz. the quantity of free water present at the highest amount of total water used in the experiments of Bouyoucos. According to the value arbitrarily assigned to this quantity, so the amount of unfree water may continually decrease with decrease of total moisture over the experimental range, or *may increase to a maximum and then decrease. It is probable that* the former is more truly representative of the actual condition in soil, although the possibility of a maximum occurring in the amount of un-

free water is suggestive in any consideration of the "optimum-moisture-content," and the passage, according to Cameron and Gallagher, of various physical properties through a maximum or minimum value at that point.

(5) The same general conclusions on the relations existing between the soil and its moisture content that were drawn from the writer's experiments on the evaporation of water from soil, follow again. The soil colloids must be considered as primarily concerned in the relations; the water present is subjected to the same law over the whole experimental range and the various constant and critical points shown by soil at varying degrees of water contents, are approximate equilibrium values only and do not indicate any break or abrupt change in the physical condition of the soil moisture.

(*Received* 1*st June*, 1919.)

THE USE OF POPPY SEED CAKE AS A CATTLE FOOD AND ITS EFFECT ON YIELD OF MILK AND COMPOSITION OF THE BUTTER FAT.

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Introduction.

Wherever the opium poppy is cultivated its seed forms a valuable secondary crop. India has a bigger outturn of seed than any other country. In the years immediately preceding the war the bulk of the poppy seed produced in India was exported. The following Table[1] gives an idea of the proportion of the total production exported for a number of years past.

Table I.

Year	Area under cultivation in British India	Estimated yield	Quantity* Exported
	Acres	Tons	Tons
1904–05	612,000	122,400	65.800
1910–11	383,000	76,600	43,400
1911–12	220,000	44,000	34,900
1912–13	197,000	39,400	23,400
1913–14	170,000	34,000	19,000
1914–15	179,000	35,800	7,000
1915–16	181,000	36,200	6.900

* Includes the seed obtained from "Malwa" opium crop, of which certain quantities were exported.

The next Table[2] (II) shows the countries to which the seed was exported:

Table II.

Principal countries of destination	1911–12	1912–13	1913–14	1914–15	1915–16	1916–17
	Tons	Tons	Tons	Tons	Tons	Tons
United Kingdom	—	—	—	84	143	—
France ...	19,400	17,800	10,700	4,175	6,635	5,253
Belgium* ...	10,200	2,900	4,800	1,360	—	—
Germany ...	4,560	2,400	3,300	· 960	—	—
Total export	34,943	23,402	18,980	6,992	6,872	5,524

* Probably largely re-exported to Germany. Figure not available.

[1] *Bull. Imp. Inst.* 1917, **15**, 3, p. 392.

[2] *Loc. cit.* p. 390.

It will thus be seen that France took the bulk of the exported seed. Owing to the falling off in the export trade, an internal market has to be found for about 12,000 to 15,000 tons of poppy seed. The seed in India is frequently eaten as such as it has a pleasant taste. Certain quantities are fed to cattle, and fairly large quantities are used in sweet-meat making and in the preparation of certain Indian foods. But it is unlikely that the demand for seed in this direction will increase sufficiently to use the whole of the production.

There seems little difficulty in finding a market for poppy seed oil in India. It is a drying oil, in some respects very similar to linseed oil. At present it fetches a rather higher price than linseed oil. It is said to be in demand in Bombay for the manufacture of certain classes of paints. In any case it is used in Europe for the preparation of white paints for artists[1]. We believe, however, that it is being largely used to adulterate ghee[2]. In Europe it was largely used as a salad oil and it is of interest here to note that at one time it was much used in Europe to adulterate olive oil[3].

A large Indian oil mill has stated that it would undertake to crush any quantity of the seed provided it could be supplied at Rs 4/4/- per maund (1 maund = 82·2 lb.; Rs 1/- = 2*s.* 0*d.* Oct. 1919).

The supply of oilcake in India seems in excess of the demand at present and much oilcake of all kinds is being used by the mills as fuel. We are proposing to test the manurial value of the cake in the coming cold weather, but it is hardly likely to compete with castor cake for this purpose and castor cake is now selling at about Rs 1/8/- per maund. The question of its use as a foodstuff has arisen and we were asked by the Opium Agent, Ghazipur, if we could undertake feeding trials. Smetham[4] gives the following analyses of poppy seed cake and meal:

	Cake per cent.	Meal per cent.		Cake per cent.	Meal per cent.
Water	10·15	9·95	Digestible Carbo-hydrates	20·04	16·24
Albuminoids	35·38	39·50	Woody fibre	7·90	9·43
Oil ...	11·43	12·13	Mineral matter	15·10	12·75

There seems to be an opinion in some quarters that the cake is not a good foodstuff. It is said by some to make animals sleepy. Kellner's

[1] Lewkowitsch, *Chem. Tech. of Oils, Fats and Waxes*, **11**, p. 477.

[2] Ghee is clarified butter and is obtained by heating butter till the greater part of its moisture is evaporated.

[3] Parry, *Foods and Drugs*, **1**.

[4] *Bulletin of the Imperial Institute*, 1917, **15**, No. 3, p. 392.

Scientific Feeding of Animals remarks that poppy seed cake is bad for milch or young cattle or pregnant animals, but that it is suitable for fattening stock. We have been unable to find any actual experimental evidence in support of these statements. On the other hand, the cake seems to be well esteemed for milch animals in the Unao District of the United Provinces. The literature goes to show that the seeds are quite free from alkaloids[1]. Moreover, Thorpe[2] states that by far the greatest part of poppy seed oil is used for edible purposes. This would point to the absence of any deleterious substance in the seed.

Attention may here be drawn to another feeding stuff derived from a drug plant (viz. hemp seed cake). During the course of investigations on the effect of feeding on the composition of milk and butter undertaken by Cranfield[3] and by Cranfield and Taylor, it was shown that the composition and quality of milk and butter produced by hemp seed cake was practically equal to that obtained by feeding linseed cake.

Dairy cattle when well fed will yield a greater amount of milk than when maintained on a lower plane of nutrition. But the quality of the milk is likewise affected to a certain extent by the feeding. For example, when a cow is changed suddenly from a grain ration to grass[4] a very marked taste is developed in the milk. Cranfield[3] showed that the effect of removal of cows from a poor pasture to a well-balanced ration results in an increase of the milk, attended with a large fall in the percentage of fat and certain other changes in the composition of fat. Again Hansen[5] found that palm oil cake in the ration increases the fat content of the milk but does not affect the yield of milk. It is also a matter of experience that linseed cake in the food produces soft butter and cotton cake and cereal grains produce a hard butter.

[1] Muller, "Alkaloids von Papaver somniferum." *Archiv der Pharmacie*, 1914, **252** (4), p. 292.

[2] *Dict. of Applied Chem.* vol. **4**, art. "Opium Seed Oil," p. 334.

[3] Cranfield, "Effect of feeding with cocoanut cake and linseed cake on the composition of butter fat." *Analyst*, **36** (1911), p. 445

Cranfield and Taylor, "Effect of feeding on composition of milk and butter; linseed cake and hempseed cake." *Ibid.* **40** (1915), p. 433; "Ditto. Dried yeast and decorticated cotton meal." *Ibid.* **41** (1916), p. 240. Cranfield, ditto, "decorticated groundnut cake and decorticated cotton cake." *Ibid.* p. 336.

[4] Eckles, *Dairy cattle and milk production*, p. 255.

[5] Hansen, "The effect of palm oil cakes upon milk production in cows." *Handw. Jahrb.* **47** (1914), p. 1, abs. in *Expt. Sta. Rec.* **33** (1913), p. 674.

EXPERIMENTAL.

Forty-nine maunds[1] of seed costing Rs 5/-[2] per maund were crushed in ordinary wooden *kolhus* or country mills at Bahraich and the total production of oil and cake was sent to us at Cawnpore. A certain amount of oil leaked out of the receptacles on the journey, but 17 mds. 10 srs. of oil was received at Cawnpore. This was sold at Rs 18/- per maund, there being a good demand for it. A bid of Rs 18/10/- per maund was made after we had closed with the offer of Rs 18/-. The cost price of the seed was thus Rs 245/- and crushing charges at Rs 1/4/- per maund amounted to Rs 61/4/-, bringing the total cost to Rs 306/4/-. The oil realised Rs 308/15/-. Thirty maunds of cake were received and the value of this represents the profit on the transaction. Of course there were charges for carriage amounting to about Rs 30/- but the seed could equally as well have been purchased and crushed in Cawnpore.

Our experiments were designed to see if we could detect any noxious property in poppy seed cake and not to test its actual food value. The latter can only be done by extended trials such as the Danish feeding trials involving the use of large herds of animals. For this we had neither the animals nor the facilities.

One cow (Phulia) and two buffaloes (Rukminia and Lachminia) were chosen for the experiment. The animals were all in good condition. The cow was of the country breed known as Kosi and the buffaloes belonged to the Murrai breed. Phulia had had her eighth calf (date of calving, 13th March, 1918). Rukminia and Lachminia had had their fourth and third calves respectively (dates of calving, Rukminia, 28th November, 1917, and Lachminia, 28th May, 1918). Rukminia was thus at the end of her lactation period and dried off during the last week of July.

The buffaloes received a daily ration of 20 lb. straw, 6 lb. bran, 3 lb. *chuni* (the outer husks and broken pieces of the pulses) and 3 lb. mustard cake. The cow's feed consisted of 13 lb. straw, 6 lb. bran, 2 lb. *chuni* and 2 lb. mustard cake. The animals were also allowed to graze. From the 1st July the supply of the mustard cake was stopped and an equal amount of poppy cake substituted instead. The feeding of poppy cake was continued for a little over six weeks. On the 14th August the poppy cake in the ration was replaced by mustard cake.

The cattle were weighed in the beginning of June while still on a

[1] 1 maund = 82·2 lb., 40 seers = 1 maund.

[2] 1 rupee = 2*s*. 0*d*. (Oct. 1919).

mustard cake ration; their weights were again taken just before discontinuing the poppy cake. The following result was obtained.

TABLE III.

Weight of animals.

	On 5th June.	On the 12th August.
	lb.	lb.
Cow Phulia	756	728
Buffalo Rukminia	952	952
„ Lachminia	1022	1008

It will be seen that the first and the third animals suffered small diminutions of 28 lb. and 14 lb. respectively in their weights and that the weight of the buffalo Rukminia remained the same during the course of the investigation. Bearing in mind the fact that the experiment was conducted during summer (which was this year even hotter than usual), the very small decrease of weight in the case of the above two animals cannot be reasonably attributed to the feeding of the poppy cake.

In fact the condition of the animals was not found to be affected in any way by the changes of food. There was no indication of the reputed action of poppy cake in bringing about drowsiness. The quality of the milk and of the butter made therefrom remained the same during the whole course of the experiment. No special taste or odour could be detected in these products while the cattle were on the poppy cake ration.

The yield of milk and its content of butter fat (by Gerber test) were recorded morning and evening for each animal.

During the latter part of the experiment the mixed milk of the whole herd of cows was included in the study as a check. These cows received the same ration as Phulia except that they received mustard cake all the time and no poppy seed cake.

Table IV shows the yields of milk and the percentage of fat in the milk.

Average daily yield of milk. These figures are graphically entered in Chart I. The conditions of weather during the course of the experiment are recorded in Chart II, as there are indications of climatic influences reacting on the yield and quality of milk. Generally speaking a fall of rain is followed by a rise in the yield of milk a few days later. This is no doubt due to the action of rain, besides bringing some comfort to the animals, on freshening up the growth of grass and thus ensuring a better pasturage.

The general fall in the milk yield during the second and third weeks of August seems to be correlated with the spell of dry weather during the first fortnight of the month. This fall in the milk yield is also noticed in the herd of cows which got a ration of mustard cake throughout.

TABLE IV.

Yield of Milk and its Fat Content.

	Cow Phulia		Buffalo Rukminia		Buffalo Lachminia		Cows' milk (mixed)	
	Average daily yield of milk in lb.	Average % fat in milk	Average daily yield of milk in lb.	Average % fat in milk	Average daily yield of milk in lb.	Average % fat in milk	Average daily yield of milk in lb.	Average % fat in milk
1918	Mustard cake period							
1 to 3 June	10·5	3·6	10·8	6·9	12·3	5·3	—	—
4 „ 6 „	11·3	3·2	9·9	6·6	13·4	5·4	—	—
7 „ 10 „	10·8	3·4	10·8	6·6	12·6	5·4	—	—
11 „ 14 „	10·1	3·4	10·6	6·9	13·1	5·1	—	—
15 „ 18 „	10·9	3·5	10·3	6·9	14·1	5·4	—	—
19 „ 22 „	10·6	4·3	9·3	6·8	13·9	5·5	—	—
23 „ 26 „	10·5	4·1	9·9	6·8	13·1	5·9	—	—
27 „ 30 „	9·1	3·6	10·1	6·9	14·8	5·8	—	—
	Mustard cake stopped; poppy cake begun							
1 „ 4 July	9·4	3·4	10·4	7·0	14·9	5·4	—	—
5 „ 8 „	10·1	3·6	10·8	7·1	15·8	5·9	—	—
							Fed mustard cake throughout	
9 „ 12 „	10·0	3·6	10·2	6·7	15·6	5·7	11·7	4·5
13 „ 16 „	8·3	3·6	8·2	7·3	15·6	5·9	11·5	4·4
17 „ 20 „	8·8	3·9	6·5	7·2	15·2	5·9	11·5	4·4
21 „ 24 „	9·8	4·5	4·9	8·0	16·4	6·1	11·7	4·6
25 „ 28 „	8·7	4·9	2·4	8·9	15·8	6·0	11·3	4·3
29 July to 1st Aug.	8·5	5·0	(Dried off)		16·8	6·1	11·2	4·9
2 to 5 Aug.	8·7	4·9	—	—	15·0	6·7	11·1	4·9
6 „ 9 „	8·4	5·0	—	—	14·1	6·9	10·8	4·7
10 „ 13 „	8·2	5·0	—	—	15·6	6·9	10·3	4·9
	Poppy cake stopped; mustard cake resubstituted							
14 „ 17 Aug.	8·4	4·2	—	—	13·2	6·3	10·8	4·9
18 „ 21 „	9·1	4·8	—	—	14·4	6·2	11·0	4·6
22 „ 25 „	10·1	5·0	—	—	15·0	6·5	11·0	4·8
26 „ 29 „	10·0	4·5	—	—	15·5	6·5	11·3	4·5
30 Aug. to 2 Sept.	10·3	4·6	—	—	16·2	6·4	11·2	4·4

The cow Phulia yielded on an average 10·2 lb. milk per day during the first period of the experiment (feeding on mustard cake). She produced 9·6 lb. milk daily during the second period (poppy cake feeding) and 10·2 lb. at the third period.

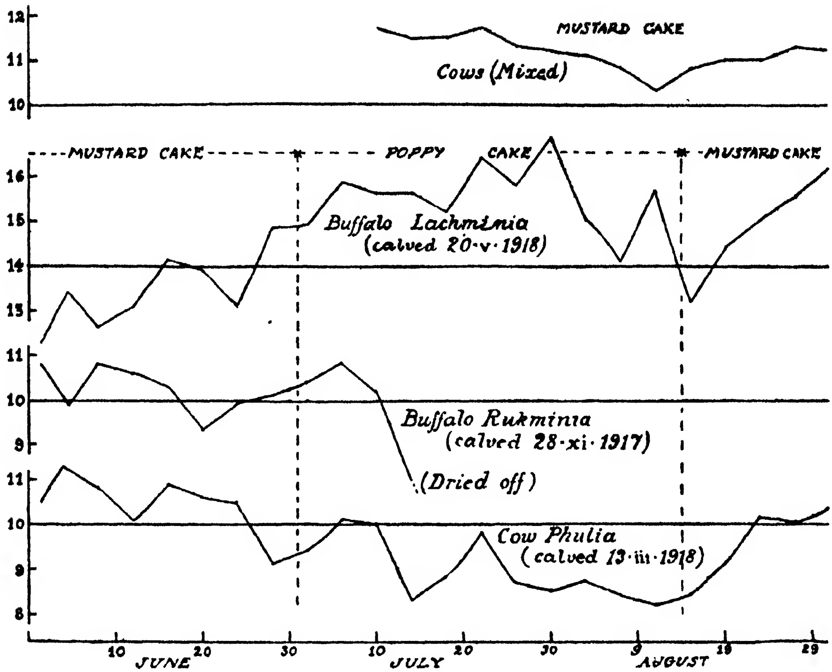

Chart I. Yield of milk in lb.

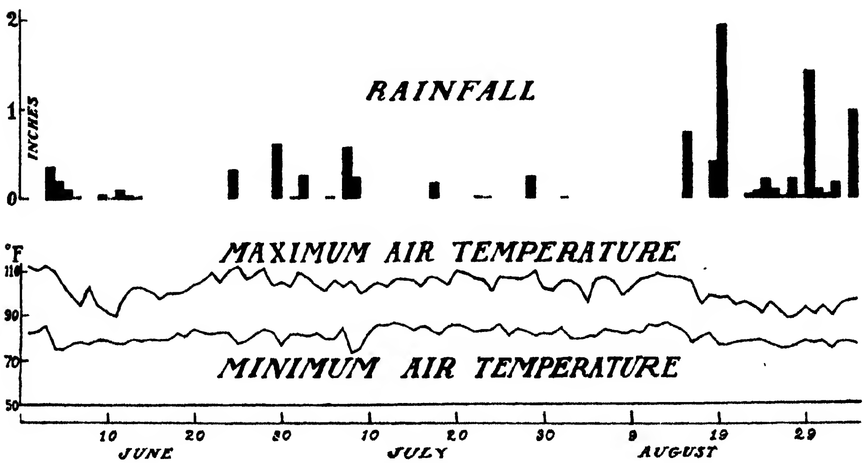

Chart II. Weather conditions.

The buffalo Rukminia yielded 10·5 lb. of milk per day at the first period and 7·5 lb. at the second period during the course of which she dried off.

The yields of milk by Lachminia were 13·5, 15·3 and 14·9 lb. respectively during the three periods of the experiment. The general tendency towards a rise in the curve of the milk yield of Lachminia is apparently due to the progress in lactation. The tendency towards a fall in Phulia's curve seems to be due to the same cause. Milk yield generally rises for the first two months after calving, after which it falls more or less regularly until the end of the lactation period[1].

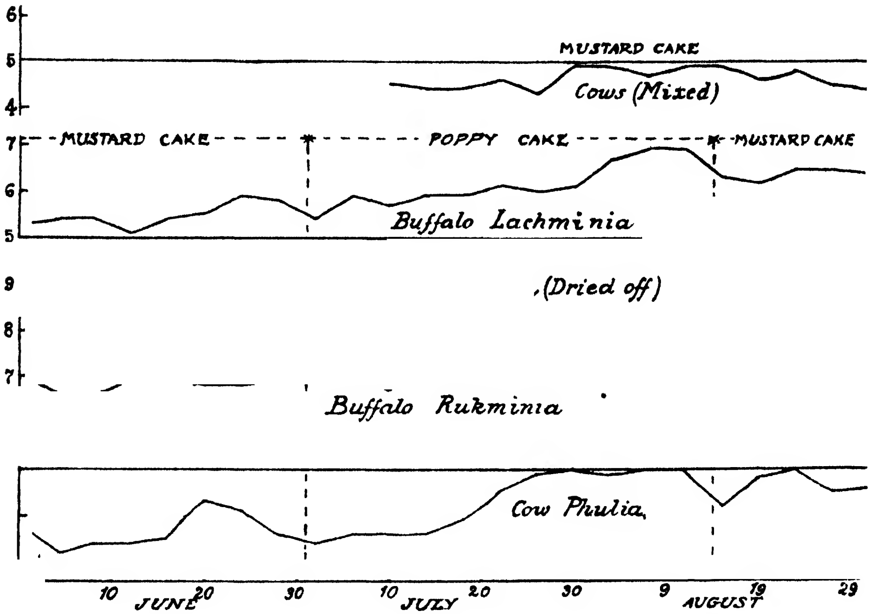

Chart III. Percentage of fat in milk.

The herd of cows (on mustard cake) produced 11·2 lb. milk per day per animal.

Judging the records as a whole the feeding of poppy cake does not seem to injuriously affect the milk yield.

Percentage of fat in the milk. This is shown in Chart III. The average fat contents in the milks of cow Phulia and buffalo Lachminia were 3·6, 4·3, 4·6 and 5·5, 6·1, 6·4 per cent. during the three periods respectively. Rukminia's milk contained 6·8 per cent. during the first and 7·5 per

[1] Berry, "Yield and composition of cow's milk during lactation." *West of Scot. Ag. Col. Bul.* **76**; abs. in *Experiment Station Record* **37** (1917), p. 373.

cent. in the second period. As is well known, the fat content shows a gradual rise with advance in the lactation period. Poppy cake, therefore, does not seem to have any deleterious effect on the fat content of milk. On comparing Charts I and III it will be seen that there are several good illustrations showing how percentage of fat rises with decreased yield of milk and how it again falls with increase in yield. It is particularly marked in the case of cow Phulia whose curves for milk yield and its percentage of fat are practically reciprocals of one another. The same seems to be the case for buffalo Rukminia.

Composition of butter fat. Besides affecting the yield of milk the food also influences the composition of butter fat. It is stated[1] that the feeding of beet-root leaves increases the proportion of volatile to non-volatile fatty acids (Reichert-Meissl and Polenske numbers). A ration of cocoanut cake also raises the Polenske number[2]. The presence of cotton seed oil can be detected in the butter within 12 to 36 hours of its being fed to cattle[3]. Cotton seed meal when fed to the cattle affects the quality of the butter, raising the melting point and lowering the Reichert-Meissl and saponification values[4].

Every third day throughout the experiment, except where otherwise stated, the cream was separated from a portion of milk. The cream was allowed to ripen and afterwards churned in a small glass churn. The butter produced was melted with the usual precautions and the following determinations made in the fat: Zeiss Butyro-refractometer values (at 40° C.), the Reichert-Meissl number, the Polenske number and the saponification value.

The following table (Table V) exhibits the data obtained.

Butyro-refractometer readings. The analytical figures obtained are graphically represented in Chart IV. The average Zeiss Butyro-refracto-

[1] Siegfield, "Influence of feeding with cocoanut cakes and beetroot leaves on the composition of butter fat; especially as regards Polenske and Reichert-Meissl values of the same." *Chem. Zeit.* **31**, 1907, p. 511; *Zeits. Unters. Nahr. und Genussm.* **13** (1907), p. 513; abs. in *Analyst,* **32** (1907), p. 256.

Alleman, "Influence of fertilizing and feeding on the milk constituents." Molk, *Ztg.* **27** (1913), p. 1666; abs. in *Expt. Sta. Rec.* **30** (1914), p. 475; and Boes and Weyland, "Influence of sugar beet feeding on the composition of the milk fats," *Zeits. Unters. Nahr. und Genussm.* **29** (1915), p. 473; abs. in *Expt. Sta. Rec.* **33** (1915), p. 674.

[2] Cranfield, *loc. cit.*; Siegfield, *loc. cit.*

[3] Smith, Wells and Ewing, "Changes in the composition of butter fat produced by feeding cotton seed oil"; abs. in *Expt. Sta. Rec.* **35** (1916), p. 775.

[4] Eckles and Palmer, "Effect of feeding cotton seed products on the composition and properties of butter." *Missouri Sta. Res. Bull.* **27**; abs. in *Expt. Sta. Rec.* **37** (1917), p. 72.

TABLE V. *Composition of Butter Fat.*

Date	Cow Phulia				Buffalo Rukminia				Buffalo Lachminia				Cows' (mixed)			
1918	Refractometer readings	Reichert-Meissl Nos.	Polenske Nos.	Saponification values	Refractometer readings	Reichert-Meissl Nos.	Polenske Nos.	Saponification values	Refractometer readings	Reichert-Meissl Nos.	Polenske Nos.	Saponification values	Refractometer readings	Reichert-Meissl Nos.	Polenske Nos.	Saponification values
					Fed mustard cake											
June 13	42·5	27·3	1·9	210·6	43·1	28·2	1·0	208·8	41·6	36·6	3·1	219·1				
„ 16	41·6	27·3	1·8.	215·0	43·1	30·1	1·3	210·9	41·2	38·8	1·9	222·4				
„ 20	41·9	27·0	2·0	215·6	43·2	28·0	1 5	213·2	40·9	36·1	1·9	221·7				
„ 24	43·0	26·8	1·9	221·0	—	—	—	—	—	—	—	—				
„ 28	42·5	28·1	1·5	226·5	43·8	25·0	1·1	219·0	42·0	33·6	1·4	226·9				
„ 30	42·4	28·2	1·9	226·8	43·3	27·6	1·2	221·3	41·5	35·4	1·5	227·8				
	Mustard cake stopped; poppy cake begun															
July 3	42·5	27·3	2·0	227·2	42·9	27·3	1·0	223·1	41·5	35·6	1·7	231·7				
„ 6	41·1	27·7	2·0	230·1	43·0	27·3	1 0	224·3	41·0	35·9	2·1	233·4				
„ 9	41·7	26·6	2·1	229·9	43·4	26·2	1·0	222·8	41·2	36·1	1·6	233·3	Fed mustard cake throughout			
„ 12	41·9	25·9	2·4	229·5	43·4	25·5	2·3	223·9	40·8	36·0	1·3	230·0	42·1	28·0	1·6	225·1
„ 15	41·1	27·9	2·4	226·7	43·9	24·4	1·0	219·5	41·5	36·3	1·7	228·0	42·1	29·3	1·7	226·0
„ 18	40·4	28·6	2·1	227·2	43·7	25·1	2·1	219·7	40·8	37·5	1·3	231·2	41·9	29·5	1·2	225·4
„ 22	41·9	26·2	1·8	224·3	43·9	24·8	1·2	220·1	41·0	35·2	1·9	233·1	41·6	30·2	2·2	229·4
„ 24	—	—	—	—	43·5	25·2	1·6	220·5	40·6	37·0	1·8	231·6	41·3	29·5	1·8	229·1
„ 25	39·7	31·2	1·8	229·9	—	—	—	—	—	—	—					
„ 28	40·5	28·5	1·7	229·8	42·8	26·1	1·4	225·7	39·6	40·9	1·8	239·7	41·5	29·0	1·8	221·5
„ 30	39·8	30·3	1·9	227 6	—	—	—	—	39·8	39·7	1·8	235·6	41·7	30·3	1·9	222·3
Aug. 2	39·4	31·3	2·1	226·8	—	—	—	—	39·9	39·7	2·0	234·8	41·3	30·4	1·9	223·9
„ 5	40·3	29·4	1·8	227·9	—	—	—	—	39·9	36·6	2·1	232·9	41·7	29·1	1·5	224·4
„ 8	40·0	29·8	1·7	229·3	—	—	—	—	39·5	38·9	1·6	234·6	41·9	29·1	1·8	223·5
„ 11	40·0	29·4	2·0	230·5	—	—	—	—	39·7	38·0	1·7	234·4	41·4	29·2	2·0	228·7
	Poppy cake stopped; mustard cake resubstituted															
„ 15	39·3	30·8	2·2	228·8	—	—	—	—	39·5	37·4	1·7	231·8	41·6	29·0	1·7	225·8
„ 19	40·6	29·5	2·3	227·4	—	—	—	—	39·3	37·5	1·7	230·0	42·0	28·9	1·6	225·9
„ 23	40·7	29·9	2·5	227·5	—	—	—	—	40·8	36·1	1·8	229·1	42·0	29·3	1·9	226·7
. 26	40·6	31·0	2·5	228·5	—	—	—	—	41·2	36·1	1·9	231·8	41·6	29·0	2·0	224·4
„ 29	40·2	31·8	2·3	230·4	—	—	—	—	41·6	34·8	1·4	229·4	41·6	29·3	1·7	230·3
Sept. 2	29·9	30·9	2·5	228·9	—	—	—	—	42·3	34·3	1·5	228·0	42·0	29·3	2·0	231·2

meter figures at 40° C. for butter fat from the milk of cow Phulia was 42·3 during the first period of the experiment (mustard cake ration) which fell to 40·7 during the second period (poppy cake ration). The value dropped further to 40·2 during the last period (mustard cake re-substituted). The variation between consecutive determinations was from 0 to 2·2.

The buffalo Rukminia dried off during the second period; the average figures were the same at the first and the second periods, viz. 43·3, and the variation was 0 to 0·7.

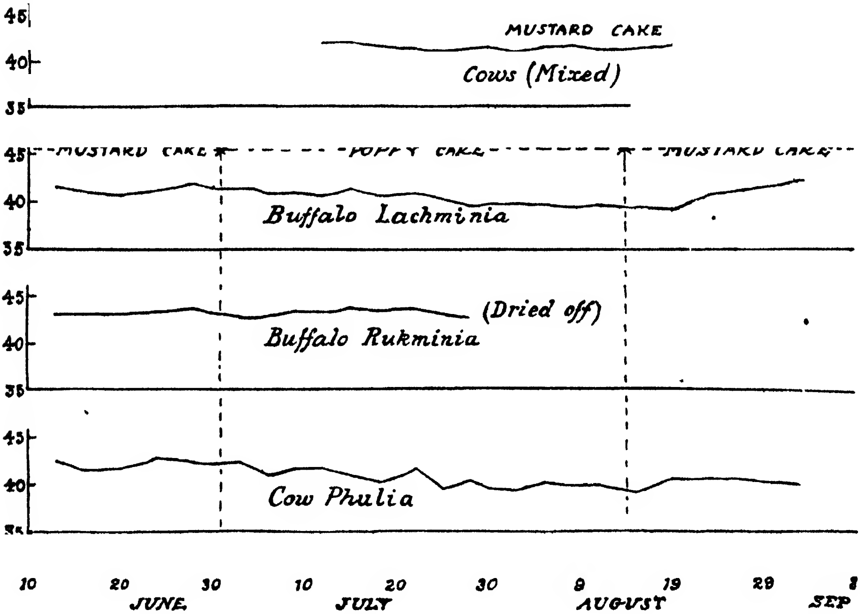

Chart IV. Zeiss Butyro-refractometer readings at 40° C.

Lachminia's figures for the three periods were 41·4, 40·4 and 40·8 respectively. The variation between consecutive determinations was 0 to 1·1.

Cows' (mixed) milk showed comparatively less variations. The average figure during the whole experiment was 41·7 (during which only mustard cake was fed). The variation was 0 to 0·5.

Reichert-Meissl numbers. These are graphically represented in Chart V. Butter from milk yielded by Phulia had an average 27·4, 28·7 and 30·6 at the three periods respectively. The variation between two consecutive determinations was from 0 to 5·0.

Rukminia's figures were 26·6 and 25·7 during the first and the second periods and the variation from 0 to 3·0. The figures for Lachminia were 36·1, 37·4 and 36·1 for the three periods and the variation was from 0 to 3·9.

The average figure for cows' (mixed) milk was 29·3 and variation was from 0 to 1·3.

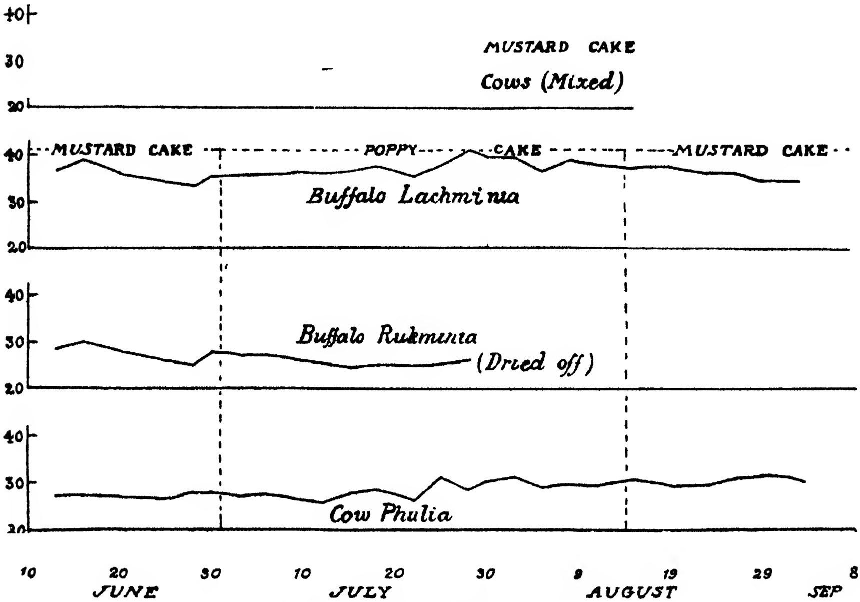

Chart V. Reichert-Meissl numbers.

A study of the changes in the values of refractometer readings and in the Reichert-Meissl numbers of two consecutive samples shows that generally speaking a fall in the refraction value is accompanied by a rise in the Reichert-Meissl number and *vice versa*. In connection with this relation between refraction and glycerides of volatile fatty acids, which has already been noted by earlier observers, it may be pointed out that the refraction increases in much greater ratio with increase of unsaturated fatty acids[1].

Polenske numbers. Chart VI shows these figures graphically. Butter fat of milk from cow Phulia had the average values 1·8, 2·0 and 2·4 for the three periods, the corresponding figures for Lachminia being 2·0, 1·7 and 1·7 respectively. The variation was 0·0 to 0·4 in the first instance

[1] Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats and Waxes*. Third edition, 2, p. 944.

and 0 to 1·2 in the second case. Rukminia's figures for the first and second periods were 1·3 and 1·4 and the variation between two consecutive determinations was from 0 to 1·3. In the case of cows' (mixed) milk the average figure was 1·8 and the variation 0 to 1·0.

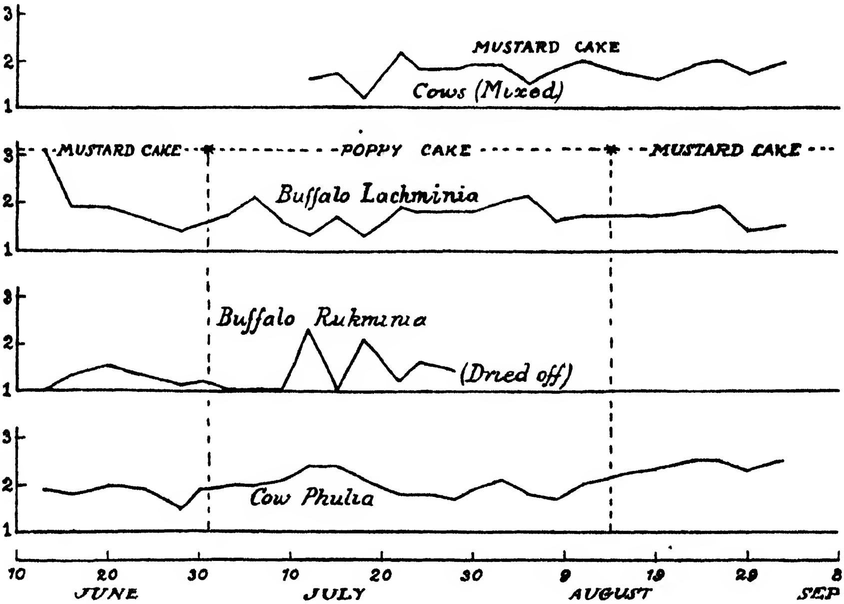

Chart VI. Polenske numbers.

Saponification values. These are entered in Chart VII. Phulia's average values for the three periods were 219·3, 228·3 and 228·6 and those for Lachminia 223·6, 233·6 and 230·0 for the three periods respectively. Rukminia's figures were 214·6 and 222·2 during the first and second periods, while butter from cows' (mixed) milk had the average saponification value of 226·1.

The curves of saponification values of the butter fat from the milk show a well-marked and fairly regular rise from the beginning of the experiment till about the end of the first week of July. This rise seems to be independent of the oil cake consumed and the period of lactation. It is found in the curves of all the three animals under experiment, and seems to depend on the climatic influences to which the animals were exposed. It may be pointed out that there was a fairly steady rise of the air temperature during the last three weeks of June.

It is of interest to observe here that the rains broke on June 3rd and

brought its usual rush of succulent green grass. It would seem likely that rise in the saponification value is connected with this.

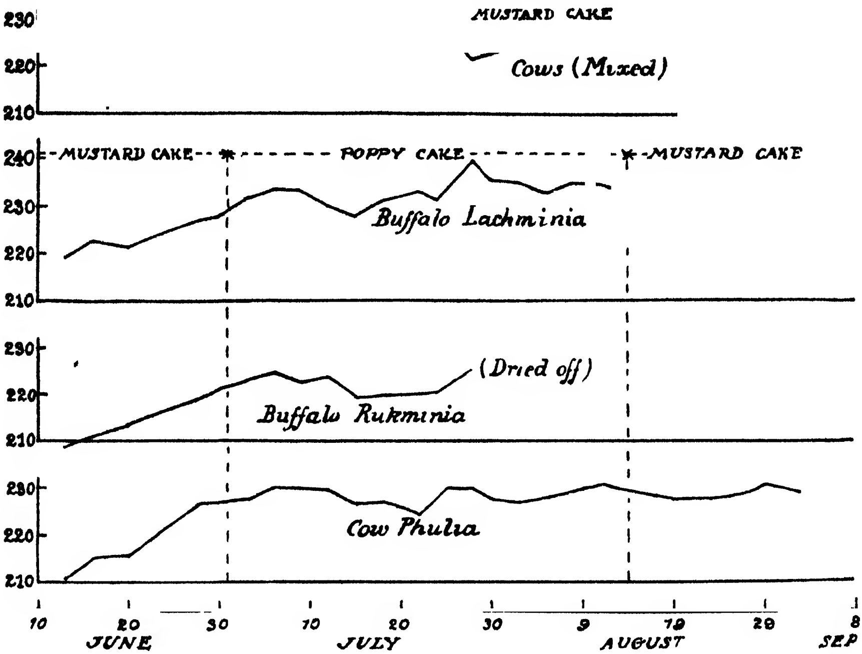

Chart VII. Saponification values.

CONCLUSIONS.

1. The substitution of poppy seed cake for mustard cake does not seem to have influenced either the yield of milk, its percentage of fat or the composition of the butter fat. The experiments were carried out on one cow and two buffaloes.

2. The reputed ill-effects of poppy seed cake in producing drowsiness and watery milk were not observed. We could detect no difference whatever in the animals when on the two different rations and the animals readily ate poppy seed cakes.

The amount of poppy seed cake fed, however, was not great.

3. The paper brings out certain indications that weather conditions influence the composition of butter fat. This may be due to indirect effects, e.g. a rush of green food after rain.

(*Received 7th June*, 1919.)

OBSERVATIONS ON SOIL PROTOZOA.

By D. WARD CUTLER.

(Rothamsted Experimental Station, Harpenden.)

Introduction.

The conclusion drawn by Russell and Hutchinson that the protozoa resident in the soil are possibly detrimental to bacterial activity, and that the beneficial results which are brought about by partial sterilisation may in part be due to the killing of these organisms, has caused a great interest to be taken in the soil protozoa, and as a consequence, a good deal of literature has been produced by various observers. Much criticism, however, has been directed against this hypothesis, some workers denying that the protozoa have any reducing effect on bacterial numbers, others asserting that these organisms are present in the soil normally as cysts, and not in the active condition.

The method of investigation, in the majority of cases, involved inoculating some medium with soil or suspension of soil, and incubating for various periods of time. By this means it can be demonstrated that numerous protozoa exist in the soil, but little or no idea is given as to whether they are present as cysts or active forms—obviously a point of great importance in its bearing upon partial sterilisation.

Martin and Lewin(1), however, showed that there was undoubtedly a trophic fauna in the soil, but they were unable to arrive at any definite conclusion as to the numbers per gram of these forms. Goodey(2), on the other hand, concludes in the case of ciliates that cysts only are present.

A systematic account of the work on soil protozoa is given by Kopeloff and Coleman(3).

There is great need therefore of a method for isolating the protozoa directly from the soil within a short period of taking the sample: but it should not involve the use of the incubator or any apparatus likely to induce excystation of those forms which were present in the cystic state.

The present investigation deals with two problems requiring solution before a suitable method can be devised for directly counting the protozoa. Firstly, an efficient and direct method of counting the number

of organisms in a unit volume of a solution is needed. Secondly, the factors governing the relation of the protozoa to the soil particles require elucidation in order to explain why it is almost impossible to find the organisms in any quantity by direct examination under the microscope, although the same soil sample can be shown to contain tens of thousands, if a dilution method is employed.

The protozoa chosen for the experiments were obtained from Broadbalk field soil, and were as follows:

Amoebae	Flagellates
A. lawesiana, Goodey	*Monas termo*
A. glebae, Dobell	*Bodo sp.*
A. sp.	*Cercomonas sp.*
	Oicomonas sp.

No attempts were made to separate these one from another and grow them in "pure" culture. Although this course presents disadvantages, the treatment of the forms "en masse" more faithfully reproduces field conditions, as these organisms are representative of the soil protozoan fauna at Rothamsted.

The average sizes of the active and cystic states are:

Active amoebae, 12·5 μ; cystic stage 10·7 μ.

Active flagellates 8·5 μ; cystic stage 4·7 μ.

The investigation on the ciliates detailed in Part II of this paper was carried out upon *Colpoda cucullus*, which measured in the active condition about 45 μ and in the cystic one from 40–45 μ.

PART I.

Method for Counting Protozoa.

Kopeloff, Lint and Coleman(4) have described a direct method for estimating the numbers of protozoa in a suspension which does not involve plating on culture media and subsequent incubation. As this seemed satisfactory it was compared with the dilution method in use at Rothamsted.

The apparatus consists of a thick glass slide in the centre of which is a hollow of depth 0·1 mm. Round this hollow is a deep groove to receive any excess fluid that may be released when a cover-glass is placed upon the slide. The hollow in the centre of the slide is divided into 625 squares, each of which is 1/25 sq. mm.

A volume of the fluid to be examined, and sufficient in amount to ensure perfect contact between the cover-glass and slide, is placed in

the hollow, and covered by the cover-glass. The preparation is then examined under the microscope, the magnification generally being approximately 600 diameters.

The protozoa in each square are then counted. Estimates are made from five samples of each solution and the results averaged. The motility of the organism is usually insufficient to cause trouble; but if it does, the fluid is first exposed to osmic acid vapour, which kills the protozoa very rapidly. Kopeloff, Lint and Coleman also suggest a method by which the organisms may be stained and killed in one process, but this I find unnecessary.

TABLE I.

Method used for counting in a suspension Protozoa whose number per c.c. is greater than 100,000.

	Squares I	II	III	IV	V	VI	VII	VIII	IX	X	Total	Average	Total No. of Protozoa per c.c. of Suspension
Sample 1	8	6	6	8	9	8	8	7	5	4	69	6·9	1,725,000
„ 2	6	5	6	7	8	9	7	6	7	8	69	6·9	1,725,000
„ 3	8	7	6	5	7	9	8	8	6	4	68	6·8	1,700,000
„ 4	9	7	6	7	4	3	5	9	9	8	67	6·7	1,675,000
„ 5	5	8	5	7	9	8	6	8	5	8	69	6·9	1,725,000
													8,550,000

Average number per c.c. of suspension, 1,710,000.

TABLE II.

Method used for counting in a suspension Protozoa whose number per c.c. is less than 100,000.

	Total number of Protozoa for 500 sq.	Total number of protozoa per c.c. of suspension
Sample 1	9	4500
„ 2	8	4000
„ 3	9	4500
„ 4	9	4500
„ 5	9	4500
		22,000

Average number per c.c. of suspension, 4400.

Two methods were employed for calculating the results.

1. The number of protozoa in ten squares is counted and the average for one square found. As one square is 0·04 sq. mm. and the depth 0·1 mm. the cubical volume is 0·004 cu. mm. The number of protozoa per cubic centimetre of the suspension is found by multiplying the average count per square by $\frac{1000}{0{\cdot}004} = 250{,}000$.

2. The total number of protozoa in 500 squares is counted. This represents an area of 500 × 0·04, that is, 20 sq. mm., or 2 cu. mm. The factor, therefore, for estimating the number per c.c. of the suspension is 2500. The two methods give concordant results: the first should be used for suspensions containing over 100,000 per c.c.; the second when fewer are present. Two typical counts are shown in Tables I and II.

The accuracy of the results was shown by checking them by a dilution method. If these two very different methods of estimation gave comparable results it seemed justifiable to assume that they were fairly accurate.

TABLE III.

Showing the results obtained by counting Protozoa in a suspension by the direct and indirect method.

Sample	Number obtained by direct method	Number obtained by dilution method: Highest dilution in which growth occurred at end of 21 days' incubation	Lowest dilution in which no growth occurred at end of 21 days' incubation
1	1,500'	1,500	1,750
2	2,500	2,250	2,500
3	4,000	4,000	4,250
4	6,500	6,250	6,500
5	10,000	10,000	12,000
6	25,400	25,000	28,000
7	35 000	33,000	36,000
8	90,500	89,000	92,000
9	143,750	145,000	150,000
10	250,000	250,000	260,000
11	537,000	535,000	540,000
12	645,000	650,000	660,000
13	885,000	880,000	890,000
14	1,059,000	1,000,000	1,100,000
15	1,258,000	1,300,000	1,400,000
16	1,500,000	1,500,000	1,600,000
17	2,300,000	2,200,000	2,300,000

10 c.c of a 1/100 dilution was made and further diluted to the necessary degrees. 1 c.c. of each dilution under investigation was then inoculated on to each of three nutrient agar plates, which were then incubated at 20° C. for 21 days, and examined at intervals. If growth of protozoa occurred on a 1/10,000 dilution plate, there must have been at least one organism to cause this growth, and hence it was assumed that there were at least 10,000 protozoa per cubic centimetre of the suspension. This method clearly gives only a minimum value, but if a series of dilu-

tions is employed varying only by small stages from one another an estimate of the numbers of protozoa can be made within narrow limits.

In Table III there are given the results obtained by the investigation of 17 suspensions, differing from one another by the degree of concentration.

Results 1–8 inclusive, by the direct method, were all obtained by counting 500 squares as described above, while the remaining results were obtained by counting ten squares and taking the average for one square.

The close similarity of the results demonstrated that the direct method was sufficiently accurate, and it was therefore employed for the work described in the second part of this paper. In order to obtain success with either method it is essential to secure uniform distribution of the organisms in the fluid. Now any large particle of a solid medium added to the suspension will render uniform distribution impossible by providing a substratum on which many of the protozoa will aggregate.

Therefore the best method of preparing the suspension is to add to the fluid successive loopfuls of the culture, each loopful being thoroughly emulsified against the side of the tube before entering the fluid. Even distribution is secured by shaking or by the successive use of a pipette.

If the organisms are found clumped together in a suspension it should be discarded.

PART II.

Factors concerned in the Relationship between the Protozoa and the Soil.

As is well known it is practically impossible to find any evidence of the presence of protozoa by direct examination of soil under the microscope, even after the necessary addition of water is made. The dilution method, nevertheless, demonstrates that these organisms are present in the soil in at least tens of thousands per gram. In a few cases protozoa have been observed by direct methods, but in numbers insignificant compared with those which must have been actually present.

Definite amounts of a suspension of amoebae and flagellate cysts were added to equal weights of different substances, the surface areas of whose particles varied one from the other, in order to test the action of these substances on the organisms.

The substances chosen were:

(*a*) Coarse sand: ignited and treated with hydrochloric acid.

(*b*) Fine sand, treated as above.

(*c*) Soil from Broadbalk wheat field.

(*d*) *Partially sterile soil* from the Broadbalk field treated for one hour with steam.

(*e*) Ignited soil.

(*f*) Rothamsted clay.

To 1 gram of each of these substances was added 2 c.c. of a suspension containing 1,645,000 amoebae and flagellate cysts per cubic centimetre. The mixtures were then gently agitated for 10 minutes, after which the solid particles were allowed to settle at the bottom of the tube, and the number of protozoa per c.c. of the supernatant fluid estimated by one of the direct methods described in Part I of this paper.

In all cases a control tube, containing the suspension but no solid matter, was tested at the end of the experiment to see whether many protozoa had sunk to the bottom of the tube: in no case was the rate of sinking sufficient to affect the experiment. As a further test, after each class of material had been investigated, the tube was vigorously shaken and another count made. In no case was there any evidence of sedimentation of the cysts apart from absorption by the solid matter.

Coarse Sand. The total number of cysts per c.c. in the supernatant fluid over the sand particles was 1,500,000: the suspension added contained 1,645,000 cysts per c.c.: the number taken up by the sand was therefore 145,000 cysts per c.c. of fluid.

Fine Sand. Under the same conditions the supernatant fluid contained 550,000 organisms per c.c.: the fine sand was therefore capable of withdrawing from the suspension 999,000 cysts per c.c.

Soil and partially sterilised soil. These two substances gave identical results; in each case 1,643,250 cysts per c.c. were taken out from the suspension.

Ignited soil. This was tried to ascertain whether the colloids of the soil were concerned in the withdrawal of protozoa from the suspension. If they are, ignition which destroys some of the colloid properties might be expected considerably to reduce the number of cysts taken up from the suspension. This actually happened, but the reduction in effectiveness was much smaller than was anticipated, for the ignited soil took up 1,501,250 organisms per c.c., or 142,000 per c.c. less than the partially sterilised or untreated soils.

Clay. In this case microscopic examination was rendered difficult by the non-settlement of the clay particles, but the estimation could still be made: 1 gram of clay withdrew from the suspension all the protozoa. A later experiment, however (Table IV), demonstrated that 1 gm.

of clay was capable of taking out of a suspension about 2,500,000 organisms per c.c.

It was often possible by careful focussing to see the cysts closely applied to the surface of the solid particles of matter. This was especially true of sand, but a similar result is obtained, though less frequently, with the varieties of soil employed.

It may be objected that during the course of these experiments many of the protozoa excysted and so caused an inaccuracy in the results. This is of course possible, and in order to test it counts were again made in the original suspension at the end of the experiment. In every case the second count was comparable with the first, the difference between the two being too small to affect the results. The following are typical of the difference in numbers obtained at these two counts: the second set of numbers is not always lower than the first, as would be the case had excystation occurred to any marked extent: the variations are within the experimental error.

At the beginning of the experiment	At the end of the experiment
550,000	560,000
885,000	880,000
1,645,000	1,650,000
1,980,000	1,990,000
2,800,000	2,775,000

In the next series of experiments the strength of the suspension was varied through wide limits. The results given in Table IV and fig. 1 show that however many flagellate and amoebic cysts are present in the suspension the number taken up by each substance is a constant, variations in different experiments being so small that they may be legitimately attributed to experimental error, and not to any variation in the power of the substances themselves. Sharp lines of demarcation exist between the various substances as regards their capacity for withdrawing protozoa from a suspension.

Experiments with Active Flagellates and Amoebae.

These experiments were carried out in nearly the same manner as the preceding, except that for greater accuracy the animals in the supernatant fluid were first killed by osmic vapour.

The results are given in Table IV: the number of active forms withdrawn from 1 c.c. of the fluid by the solid particles is similar to the number of cysts taken by the same substance.

It may be concluded therefore that the capacity of sand, soil and clay for retaining flagellates and amoebae is independent of the condition of the organisms, whether they are in the cystic or active form, but varies with the size, as experiments with ciliates demonstrate.

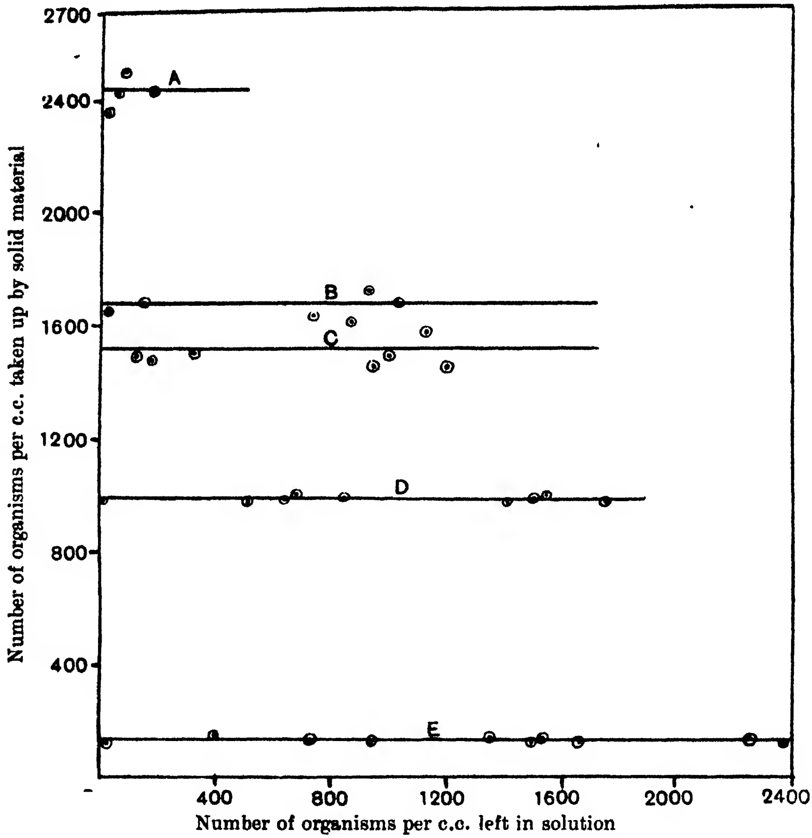

Fig. 1. Showing the number of amoebae and flagellates withdrawn from suspensions of varying strengths by the different types of solid matter. A = clay, B = untreated and partially sterilised soil, C = ignited soil, D = fine sand, E = coarse sand. Since complete retention occurs when the number of organisms added is less than the capacity of the solid matter, the first part of each of the above curves is coincident with the ordinate. The numbers of organisms are given in thousands.

Experiments with varying amounts of solid matter.

In these experiments 2 c.c. of suspension was added to weights of solid matter varying from 1–0·1 gram. Again the results demonstrate that solid matter has a specific capacity for withdrawing a definite

TABLE IV.

Cysts of Flagellates and Amoebae.

Strength of suspension per c.c	No per c.c. taken up by coarse sand particles, 0·1–0·2 mm.	No per c.c. taken up by fine sand particles, 0·2–0·04 mm	No per c.c. taken up by ordinary soil and partially sterilised soil	No per c.c. taken up by ignited soil	No per c.c. taken up by clay
96,800	C*	C	C	C	C
130,000	C	C	C	C	C
155,000	142,000	C	C	C	C
550,000	150,000	C	C	C	C
885,000	148,000	C	C	C	C
1,000,000	145,000	995,000	C	C	C
1,500,000	150,000	985,000	C	C	C
1,645,000	145,000	995,000	1,643,250	1,501,250	C
1,690,000	150,000	1,008,750	1,665,000	1,498,250	C
1,832,000	146,000	1,000,500	1,686,250	1,506,250	C
2,399,999	148,000	980,000	1,637,499	1,445,000	2,368,749
2,500,000		1,000,000	1,630,000	1,500,000	2,430,000
2,656,250		1,100,000	1,740,000	1,450,150	2,456,000
2,736,250		998,542	1,687,250	1,587,000	2,550,000
Active Flagellates and Amoebae.					
20,000	C	C	C	C	C
35,000	C	C	C	C	C
50,250	C	C	C	C	C
168,500	139,500	C	C	C	C
230,000	149,000	C	C	C	C
560,150	150,150	C	C	C	C
1,005,000	142,250	999,950	C	C	C
1,640,000	148,276	1,006,425	1,639,950	1,580,625	C
1,980,250	142,150	988,000	1,690,150	1,487,342	C
2,670,000	147,365	996,560	1,600,000	1,560,000	2,560,250
2,800,000	139,295	1,005,245	1,763,150	1,499,950	2,489,350
Experimental error	7 %	11 %	9 %	9 %	9 %

* In the above table C denotes that the supernatant fluid was devoid of protozoa and that therefore the solid matter had completely withdrawn them from the suspension.

number of organisms from a suspension. Thus when ·25 gram of either sand, soil or clay was employed there was retained only 1/4th of the number of organisms retained by 1 gram of the substance. For these results see Tables V and VI.

Effect of varying the Time Factor.

The preceding investigations were all performed with the time factor constant, which had been arbitrarily fixed at 10 minutes. In these final experiments this factor was varied. The results were not

affected, showing that the action between the surface particles and protozoa is practically instantaneous.

TABLE V.

Strength of suspension	Amount of solid material	Time of action	Coarse sand	Fine sand	Soil	Ignited soil	Clay
	gram						
550,000	1	F*	140,000	C	C	C	C
885,000	1	F	147,000	C	C	C	C
1,700,000	1	F		1,005,250	1,665,000	1,560,000	C
1,236,000	1	F	145,275	1,000,000	C		
1,837,500	1	5 min.	145,000	1,062,500	1,675,150	1,506,250	
1,235,000	1	2 min.	146,150	1,016,667	C		C
550,000 (killed)	1	5 min.	142,500	C	C		C
2,200,000 (killed)	1	7 min.	147,250	1,000,150	1,600,000	1,530,250	C
855,000 (killed)	1	1 min.	143,150	C		C	
2,700,000 (killed)	1	4 min.			1,625,350		2,535,000
2,975,000 (killed)	1	5 min.		1,100,000			2,435,150
575,000	0·2	3 min.	30,000	203,000	335,150		500
60,000	0·25	2 min.	36,500	C	C	C	C
325,000	0·25	F	35,000	230,000	C	C	C
1,300,000	0·5	F	74,250	500,000	815,000	780,000	1,150,000

* In the above table F indicates that the suspension was filtered through the solid material.

Effect of filtering suspension through soil.

The soil or sand was placed in the bulb of a 20 c.c. pipette, and the suspension allowed to filter through. Examination of the filtrate showed that the number of organisms retained by the solid matter was the same as in the experiments detailed above.

Further the results obtained by using a suspension of protozoa previously killed by heating at 80° C. for 5 minutes were identical with those obtained when the organisms were alive: the action is therefore physico-chemical and is not determined by any "vital factor."

In Table V are given in tabulated form the results obtained when the various factors described above are varied.

Experiments with Ciliates.

Both active and cystic forms of *Colpoda cucullus* were investigated. The procedure was that employed for amoebae and flagellates, except that the counting was always done by the 500 square method described

in Part I of this paper. Immediately before examination the fluid was subjected to the action of osmic vapour for a few seconds to kill the ciliates.

TABLE VI.

Active ciliates.

Strength of suspension	Amount of solid matter	Time of action	Coarse sand	Fine sand	Soil	Ignited soil	Clay
	gram						
10,000	1	10 min.	C	C	C	C	C
15,000	1	10 min.	C	C	C	C	C
25,000	1	5 min	C	C	C	C	C
35,000	1	F*	27,500	C	C	C	C
45,000	1	F	25,150	C	C	C	C
200,000	1	7 min.	27,000	185,000	C	C	C
400,000	1	1 min.	26,150	184,500	280,000	270,000	C
500,000	1	10 min.	28,000	190,000	280,500	270,250	450,000
45,000	0·2	5 min.	5,000	37,000	C	C	C
45,000	0·1	F		18,500	28,322	26,900	45,000

Ciliate cysts.

Strength of suspension	Amount of solid matter	Time of action	Coarse sand	Fine sand	Soil	Ignited soil	Clay
5,000	1	10 min.	C	C	C	C	C
20,000	1	10 min.	C	C	C	C	C
32,500	1	5 min.	28,000	C	C	C	C
400,000	1	F	27,500	184,000	280,150	270,000	C
500,000	1	3 min.		190,000	280,000	275,250	450,000
600,000	1	F	28,000	185,150	282,000	214,250	440,150
5,000	0·1	F	2,500	C	C	C	C
20,000	0·5	5 min.	13,500	C	C	C	C
300,000	0·25	7 min	7,000	46,500	70,000	67,250	100,000

* F indicates that the suspension was filtered through the solid material.

Experiments in which the strength of suspension, time of action, and amount of solid matter used were varied demonstrated that the different kinds of materials per gram were capable of retaining specific numbers of organisms per c.c. (see Table VI). For the sake of convenience the numbers below are given to the nearest thousand.

Coarse Sand	Fine Sand	Soil	Ignited Soil	Clay
27,000	185,000	280,000	270,000	450,000

These figures are much lower than those obtained with experiments on amoebae and flagellates, as was expected on account of the enormously greater size of the ciliate.

The ratio which one substance bears to another as regards capacity

for retaining amoebae and flagellates is practically the same as the ratio of their capacities to retain ciliates:

	Ratio of			
	Coarse Sand to Soil	Fine Sand to Soil	Soil to Ignited Soil	Soil to Clay
Amoebae and Flagellates	1 : 6·7	1 : 1·6	1 : 1·06	1 : 1·5
Ciliates	1 : 6·8	1 : 1·5	1 : 1·04	1 : 1·6

The ratio of the mean diameter of the amoebae or flagellates to that of ciliates is as 1 : 5, while the ratio of the mean volume of the amoebae or flagellates to that of the ciliates is as $1 : 5^3$. On the other hand the ratio of the holding power of the various substances used is for ciliates and amoebae or flagellates as 5 : 1 approximately. Thus the ratio of the retaining powers of the various substances is inversely proportional to the ratio of the diameter of the protozoa and to the cube root of their volumes. Some relationship between these variants seems probable, but at present it has not been discovered.

Discussion.

The foregoing results demonstrate that the factors governing the relation between soil protozoa and soil particles are largely physico-chemical and primarily of the nature of surface action. As the size of the particles diminishes so the number of protozoa retained increases, till finally 1 gram of clay withdraws 2,500,000 flagellates and amoebae from 1 c.c. of the suspension. Different types of soil probably differ in their capacities according as their content of sand or clay was high or low, for it has been shown that the results are the same if the suspension is allowed to filter through the soil as would occur in a field.

The surface action, however, between the protozoa and the soil particles appears to differ from ordinary adsorption. The action is linear up to the point when a suspension is used of a strength less than the retention capacity of the substance, then complete withdrawal of the organisms from the suspension takes place. This is in sharp contrast with adsorption, which is never complete. Also there is no similarity between a typical adsorption curve and those given in fig. 1. Nor could any be expected. Rothamsted soil is estimated to contain some 12,000 million particles per gram, possessing an area of the order of 2,500 sq. centimetres: 18 per cent. is clay with particles of 2 μ downwards; 53 per cent. is silt with particles of diameter 25–6 μ. The average diameter of the protozoa is much greater than that of the clay particles and equal or only slightly less than that of the silt particles. Thus any

attempt to regard the action between protozoa and soil particles as one of adsorption is rather hopeless, and the fact that experiment negatives such a view is not a matter for surprise.

A further point of interest at present inexplicable is that so few organisms are retained by the soil. Assuming the approximate area of 1 gram of Rothamsted soil to be 2500 sq. cm. this figure is much larger than the total area of the amoebae and flagellates retained by the soil, which is only 4·2 sq. cm. approximately. In the case of fine and coarse sand the area covered by the retained organisms is much larger, though still below what might have been expected.

Examination of the five columns in Table IV shows that as the number of particles in the material increases so also does the number of organisms taken up. It is remarkable, however, that fine sand proves so effective as compared with coarse sand, and that ignited soil has a capacity so nearly equal to that of the untreated and partially sterilised soil. The particles of fine sand appear to be of sizes most suitable for retaining the organisms. With ignited soil the power of retention is almost as great as is that of untreated soil, thus indicating, under the conditions of these experiments, that the effective agent is the surface area of the particles irrespective of their colloidal properties.

Part I of this paper showed that the dilution and direct methods are comparable. It is safe then to assume that the number of protozoa found per gram of soil by the dilution method probably represents fairly accurately the actual numbers in the soil sample. Since various observers have shown that the number of amoebae and flagellates usually present in the soil is between 10,000 and 100,000 per gram, it is evident that the number of protozoa in an average sample of soil is far less in number than the soil is capable of retaining. Russell and Golding(5) found numerous protozoa in sewage sick soils and by the use of the centrifuge they were able to obtain some of the active forms free from the soil particles. Probably the conditions were such that excessive reproduction of the protozoa occurred until the numbers were greater than the retaining power of the soil. Protozoa would then be found lying free from soil particles and would be acted upon by the centrifuge. Further investigations on these lines are in progress.

Part II of this paper demonstrates that the protozoa are normally resident on soil particles, therefore their environment may be of a different nature from that sometimes assumed. Russell and Appleyard(6) showed that the "free" air of the soil was approximately that of the atmosphere, but that there was also a second atmosphere dissolved in the colloidal

substance surrounding each particle, which was characterised by an increased percentage of CO_2 and nitrogen and the absence of oxygen. If therefore there are anaerobic protozoa in the soil, and experiments in this laboratory indicate that such is the case, this second dissolved atmosphere provides a suitable environment. Also the physical conditions of the water around soil particles may differ from those in the free spaces of wet soil: how far, however, these factors will influence the life of the protozoa requires investigation.

Finally, these experiments have a distinct bearing on the physiological condition vaguely termed "positive *thigmotaxis*," or the tendency for small living organisms to adhere to hard surfaces. This is a widespread phenomenon occurring both in plants and animals. A case is recorded by Verworn of a small ciliate--Oxytricha—which coming into contact with the egg of a rival mussel (Anodonta) remained on the surface for four hours, unable to leave it until a piece of mud drifted sufficiently near to the egg to allow escape. Jennings has also described how Paramoecia will adhere in countless numbers to a piece of filter paper introduced into the fluid in which they are living.

Also there is the well known phenomenon of the spermatozoa clustering and adhering to the egg during the process of fertilisation. This is no place to enter into the discussion of this physiological question, but it may be pointed out that the observations can be explained on surface action factors probably of a kind similar to those governing the relation between protozoa and the soil particles.

SUMMARY.

1. It has been shown that the direct counting method for soil protozoa devised by Kopeloff and Coleman for use in liquid media gives results entirely comparable with those obtained by a dilution method.

2. The factors governing the relation between the protozoa and the soil particles are those of surface action, and the capacity of various substances, sand, soil and clay, for retaining these organisms is specific and constant.

3. Coarse sand is capable of withdrawing per gram approximately 145,000 amoebae and flagellates per c.c. from a suspension of any strength. Fine sand withdraws approximately 980,000 per c.c.: soil and partially sterilised soil 1,650,000, ignited soil 1,500,000 and clay 2,450,000.

4. These figures are constant for given material and organisms and

are independent of the concentration of the suspension, the time of action, or whether the suspension contains cysts or active forms of the amoebae and flagellates investigated. Also the action is the same when the experiment is performed with a suspension of living or dead organisms.

5. Experiments with the ciliate—*Colpoda cucullus*—show that coarse sand per gram retains 27,000 per c.c.; fine sand per gram 185,000 per c.c.; soil and partially sterilised soil 280,000 per c.c.; ignited soil 270,000 per c.c. and clay 450,000 per c.c.

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(*Received* 21*st June*, 1919.)

CAMBRIDGE: PRINTED BY J. B. PEACE, M.A., AT THE UNIVERSITY PRESS.